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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
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Immunokinases

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IMMUNOKINASES

The present invention relates to a complex formed from at least one component A and at least one component B. The present invention also relates to nucleic acids and/or vectors coding for such a complex. The present invention furthermore provides a method for influencing the cell growth and/or the physiology of cells to which said complex, nucleic acids or vectors have been targeted. The invention further relates to cells or non-human organism, such as microorganisms or cell lines, producing the complex of the present invention. The present invention also concerns a kit comprising said complex, nucleic acids, vectors and/or cells. The present invention relates to the use of said complex, nucleic acids, vectors, cells or kit for the manufacturing of a medicament for the treatment of proliferative diseases, allergies, autoimmune diseases and/or chronic inflammation. The present invention further relates to the use of said complex, nucleic acids or vectors, cells and/or kit for targeted modulation of cellular signalling pathways, in order to affect the gene expression, and/or the viability of the target cell in a therapeutic manner. The invention further relates to a medicament comprising said complex, nucleic acids, vectors, cells or organisms. Furthermore the complexes, nucleic acids, vectors, cells and kits of the present invention are usable in prognostic, diagnostic and analytic kinase assays.

Background of the invention

Medications currently available for proliferative diseases, such as chemotherapeutic agents, have the disadvantage of inducing considerable side effects due to their relative non-specificity. It has been attempted to moderate these by various therapeutic concepts. One potential approach is the use of immunotherapeutic agents to increase the specificity of medication. This approach has been especially useful for the treatment of tumors.

One type of an immunotherapeutic agent are immunotoxins. An immunotoxin comprises a monoclonal antibody (moAb) or a recombinant antibody fragment with a specific affinity for surface markers of target cells, which is coupled to a cytotoxic reagent. Cytotoxic agents are selected from toxins or radioactive elements. An immunotherapeutic wherein the cytotoxic agent is a radioactive elements is called radioimmunoconjugate. Immunotoxins and radioimmunoconjugate have been used for the treatment of malignancies.

Another type of immunotherapeutic agent are anti-immunoconjugates. An anti-immunoconjugate comprises a structure relevant to pathogenesis or a fragment thereof, which is coupled to a toxin component. Anti-immunoconjugates are used for the treatment of autoimmne diseases, tissue reactions or allergies.

When radioactively labeled anti-B-cell moAb were used with B-cell lymphomas, tumor regressions and even complete remissions could be observed (1). In contrast, the results with moAb against solid tumors were rather disillusioning. The relative large size of the ITs used in these studies seemed to interfere with their ability to penetrate the tumors, and made them ineffective therapeutics. The low tumor penetration rate posed a particular challenging problem for poorly vascularized tumors. In order to obtain better tissue and tumor penetration and in general improved diffusion properties, the ITs were miniaturized. It was also speculated, that these smaller ITs would be less immunogenic because of the reduced size of the antigenic determinants (2). Therefore proteolytically cleaved antibody fragments (miniaturized) were conjugated to the above mentioned effector functions (radioactive elements or toxins).

Improved cloning techniques allowed the preparation of completely recombinant ITs: Coding regions of immunoglobulin light and heavy chain variable regions, amplified by polymerase chain reaction, are joined together by a synthetic linker (e.g. $(Gly_4Ser)_3$) (SEQ ID NO: 7). The resulting single chain fragment of variable region genes (scFv) is then genetically fused to a coding region of a catalytically active enzyme including cytotoxically active proteins or polypeptides (3).

The peptidic cell poisons which have been mostly used to date and thus best characterized are the bacterial toxins diphtheria toxin (DT), *Pseudomonas* exotoxin A (PE), and the plant-derived Ricin-A (4). The mechanism of cytotoxic activity is essentially the same in all of these toxins despite of their different evolutionary backgrounds. The catalytic domain inhibits protein biosynthesis by direct modification of the elongation factor 2 (EF-2), which is important to translation, or by inactivation of the EF-2 binding site at the 28S-rRNA subunit of ribosomes.

In most of the constructs employed to date, the systemic application of immunotoxins results in more or less severe side effects. In addition to the "vascular leak" syndrome, thrombocytopenia, hemolysis, renal insufficiency and sickness also occur, depending on the construct employed and the applied dosage (4). Dose-dependent liver damage was also observed (5). In addition to the documented side effects, the immunogenicity of the constructs is one of the key problems of immunotherapy. This applies, in particular, to the humoral defense against the catalytic domains employed, such as Ricin (HARA), PE, or DT (2). Theoretically, all non-human structures can provoke an immune response. Thus, the repeated administration of immunotoxins and immunoconjugates is limited. A logical consequence of these problems is the development of human immunotoxins.

To date, human toxins used in immunotoxins have in most of all cases been selected from ribonucleases (6). Since human RNases are present in extracellular fluids, plasma and tissues, they are considered less immunogenic when used in immunotoxins. Angiogenin (ANG), a 14 kDa protein having a 64% sequence homology with RNase A, was first isolated from a tumor-cell-conditioned medium, where it was discovered due to its capability of inducing angiogenesis (7). It was shown that the t-RNA-specific RNase activity of Angiogenin has a cytotoxic potential. In accordance with that, chemically conjugated immunotoxins subsequently exhibited a cell-specific toxic activity. To evaluate the efficacy of ANG-based immunotoxins, different conformations of ANG with, e.g. epidermal growth factor (EGF) or CD30 ligand, were constructed and successfully tested *in vitro* (8). Another member of the RNase superfamily is eosinophilic neurotoxin (EDN). For EDN, which has a size of

18.4 kDa, only the direct neurotoxicity has been described to date. Based on the documented potency, different EDN-based immunotoxins have been constructed and successfully tested *in vitro* (9).

Very recently it was shown that proteases like granzyme B or derivatives thereof can efficiently fulfill the effector function of immunotoxins (PCT/EP01/04514).

Protein phosphorylation is one of the most important mechanisms by which extracellular signals are transformed into biological responses in cells. Activation of protein kinases is the most common mode of signal transduction in biological systems. The three basic components of the phosphorylation systems are: 1) phosphoproteins that alter their properties by phosphorylation and dephosphorylation; 2) protein kinases that transfer a phosphate group from donor substrates, such as ATP and GTP, to serine, threonine, tyrosine or histidine residues; and 3) protein phosphatases that dephoshorylate phosphorylated proteins, thereby restoring the particular protein phosphorylation system to its basal stage. The eukaryotic protein kinases (ePK) represent the largest superfamily of homologous proteins that are involved in the regulation of intracellular signaling pathways. These kinases phosphorylate amino acid (aa) residues located in the loops or turns of their substrates. To regulate signal transduction pathways, there are approximately 2000 kinases and 500 protein phosphatases encoded within the human genome (10). A large number of these kinases are encoded by oncogenes and tumor-suppressor genes. The primary structures of hundreds of these enzymes are known, and all contain a conserved catalytic core of about 250-300 aa residues. The conserved structural features of the catalytic domain have been found from yeast, lower eukaryotes to mammals. The catalytic domain of a kinase domain is further divided into 12 smaller subdomains, defined as regions uninterrupted by large insertions and containing characteristic, highly conserved aa residues. Subdomain I-IV, located at the amino-terminus of the catalytic domain, is involved in anchoring and orienting the nucleotide ATP. Subdomains VI-IX form a large lobe structure at the carboxy-terminus of the catalytic domain and are involved in the binding of substrates and catalyzing the phospho-transfer reaction. The pattern of aa

residues found within subdomain VIB (HRD motif), VIII (A/SPE motif), and IX (DXWXXG motif (SEQ ID NO. 9) are highly conserved among different protein kinases.

The eukaryotic protein kinases make up a large superfamily of homologous proteins (11). A classification scheme is founded on a catalytic domain phylogeny, which reveals families of enzymes that have related substrate specificities and modes of regulation according to the scheme of Hanks and Hunter (12). Most protein kinases contain a conserved catalytic domain belonging to the eukaryotic protein kinase (ePK) superfamily (all other protein kinases are classified as atypical protein kinases (aPKs)). ePK's are classified into seven major groups, and are subdivided into families, and subfamilies, based on the sequence of their ePK domains:

Atypical protein kinases (aPK) lack sequence similarity to the ePK domains, but either have protein kinase activity, or a clear homology of aPKs with protein kinase activity. All aPK families are small, several having just one member in vertebrates. None have been found in invertebrates. A number of reports have shown that the kinases of this subfamily play critical roles in signaling pathways that control cell growth, differentiation and survival. Recently, several investigators have identified a number of aPKC-interacting proteins and their characterization is helping to unravel the mechanisms of action and functions of these kinases. Recently, a new family of aPKs called alpha kinases that does not have any homology to the serine/threonine/tyrosine protein kinase superfamily has been identified (13). The alpha kinases differ from serine/threonine/tyrosine protein kinases in that that they phosphorylate a threonine aa residue located in the alpha helical region of the substrate.

Free calcium is a major second messenger in all cell types. One mechanism by which calcium ions exert their effects is by binding to a 17-kDa protein, calmodulin (CaM). The binding of four calcium ions to calmodulin changes its conformation and promotes its interaction with a number of other proteins, including several classes of protein kinases that are activated by the calcium/CaM complex (14). Classifying the calcium/CaM-dependent protein kinases is based on their substrate specificity. Some of these enzymes have

only one substrate, and are designed as *"dedicated"* calcium/CaM-dependent protein kinases, while others have broad substrate specificity and are termed *"multifunctional"* kinases. The *dedicated* calcium/CaM-dependent protein kinases comprise three enzymes. Phosphorylase kinase, myosin light chain kinase and eukaryotic elongation factor-2 kinase. Multifunctional calcium/CaM-dependent protein kinases comprise four enzymes referred to as CaM-kinases I, II, IV and pro-apoptotic serine/threonine death protein kinases.

One of the positive mediators of apoptosis is DAP-kinase (DAPk) (15). DAPk is a pro-apoptotic calcium/CaM-regulated serine/threonine kinase with tumor-suppressive activity. DAPk is frequently inactivated by promoter methylation in human cancer. Its expression is frequently lost in human carcinoma and B- and (NK)/T-cell malignancies, in some cases in association with more aggressive stages of disease (16). Very recently, it has been shown, that no expression of DAPk was detectable in high-metastatic lung carcinoma cell lines, whereas the low-metastatic counterparts were positive for DAPk. Four additional kinases that have a significant homology in their catalytic domain to DAPk were recently identified. ZIP(Dlk)-kinase and DRP-1, also named DAPk2, are the closest family members, as their catalytic domains share approximately 80% identity to that of DAPk. Two more distant DAPk-related proteins are DRAK1 and DRAK2. Both the pro-apoptotic and tumor-suppressive functions of DAPk depend on its kinase catalytic activity. The CaM-regulatory segment of DAPk possesses an autoinhibitory effect on the catalytic activity, and is relieved by binding to Ca2+ -activated CaM. Consistently, the deletion of this segment from DAPk-ΔCaM mutant generated a constitutively active kinase ("super-killing kinase"), which displayed CaM-independent substrate phosphorylation *in vitro* and promoted apoptotic activity in *vivo* (17). Eukaryotic elongation factor-2 kinase (eEF-2k) belongs to the alpha kinases and is distinct from the main family of protein kinases with which they share no sequence similarity (18). The activity of eukaryotic elongation factor-2 (eEF-2) is crucial for the elongation step of mRNA translation. eEF-2 activity is regulated by phosphorylation. To be active, eEF-2 must be dephosphorylated, since phosphorylation at Thr-56 and 58 causes inactivation, resulting in the termination of mRNA translation. Phosphorylation of eEF-2 at

Thr-56 and 58 by the highly specific calcium/CaM-dependent eEF-2k results in eEF-2 inactivation and, therefore, may regulate the global rate of protein synthesis at the elongation stage in animal cells. eEF-2k is itself regulated both negatively and positively by phosphorylation on at least five different serine residues, probably mediated by seven or more protein kinases. Very recently, it has been shown, that a point mutation at Ser-499, eEF-2K S499D, transforms the kinase into a constitutively active form (19).

Protein phosphorylation is implicated in cellular processes such as proliferation, differentiation, secretion, invasion, angiogenesis, metastasis and apoptosis. Protein kinases and phosphatases play key roles in regulating these processes. Changes in the level, subcellular location and activity of kinases and phosphatases have consequences on normal cell function and maintenance of cellular homeostasis. Dysfunction in activities of protein kinases may lead to severe pathological states. In cancer, as well as in other proliferative diseases, deregulated cell proliferation, differentiation and survival frequently results from abnormal protein phosphorylation.

The identification of the key roles of protein kinases in proliferative diseases has led to extensive efforts to develop kinase inhibitors for treatment of a wide range of cancers. Many different tyrosine and serine/threonine protein kinases have been selected as candidates for drug discovery activities in oncology/inflammatory research, based either on their overexpression and/or on dysfunction in a particular organ or tissue, or through their association in deregulated signal transduction/cell cycle pathways. To date, more than 30 different tyrosine kinase targets are under evaluation in drug discovery projects in oncology. Chemical inhibitors (organic molecules, peptide inhibitors), antisense oligonucleotides and kinase-selective antibodies have been developed which target intracellular kinases.

Nevertheless, development was slow and associated with problems, mainly because of the associated toxicity, attributed to the poor selectivity of these compounds. Protein kinase inhibitors mainly bind at the active site of the enzyme, in competition with ATP+, and whether such inhibitors could ever be used for the long-term treatment of chronic conditions, such as rheumatoid arthritis, is still questionable.

Similarly the state of the art immunotoxins, such as chemically-linked or recombinant immunotoxins comprising ribonucleases, are still associated with the problem of unspecific toxicity. This problem reduces the efficiency of compositions comprising said immunotoxins, and limits their usefulness as therapeutic agents.

Surprisingly it was found that the above-mentioned problems can be solved by constructing complexes comprising cell-specific antibody fragment(s) which is/are linked to catalytic active kinase(s) that develop cytotoxic/regulative activity upon internalization of the complex. Surprisingly, the complexes of the present invention are superior over state of the art immunotoxins in that they have a reduced immunogenicity, an improved activity and are resistant to non-specific inactivation, and are thus are less prone to activity reduction.

Summary of the invention

The present invention concerns a complex formed from at least one component A and at least one component B, whereby component A has a binding activity for cellular surface structures, and component B has kinase properties. The component A is selected from the group of actively binding structures consisting of antibodies or their derivatives or fragments thereof, and/or chemical molecules such as carbohydrates, lipids, nucleic acids, peptides, vitamins, etc., and/or small molecules with up to 100 atoms with receptor-binding activity such as ligands, in particular single atoms, peptidic molecules, non-peptidic molecules, etc., and/or cell surface carbohydrate binding proteins and their ligands such as lectins, in particular calnexins, c-type lectins, l-type lectins, m-type lectins, p-type lectins, r-type lectins, galectins and their derivatives, and/or receptor binding molecules such as natural ligands to the cluster of differentiation (CD) antigens, like CD30, CD40, etc., cytokines such as chemokines, colony stimulating factors, type-1 cytokines, type-2 cytokines, interferons, interleukins, lymphokines, monokines, etc., and/or adhesion molecules including their derivatives and mutants, and/or derivatives or combinations of any of the above listed of actively binding structures, which bind to CD antigens, cytokine receptors,

hormone receptors, growth factor receptors, ion pumps, channel-forming proteins. The component A may also be selected from the group of passively binding structures consisting of allergens, peptidic allergens, recombinant allergens, allergen-idiotypical antibodies, autoimmune-provoking structures, tissue-rejection-inducing structures, immunoglobulin constant regions and their derivatives, mutants or combinations thereof. The complex of the present invention is directed by its component A to a target cell comprising a binding partner for the above listed binding structures of A. In a further embodiment the component A of the complex has a higher valency by comprising two or more identical and/or different binding structures. The complex of the present invention also comprises a component B which is at least one kinase selected from the following three classes of kinases: 1. eukaryotic protein kinase (ePK) superfamily, 2. histidine protein kinase (HPK) superfamily or 3. atypical protein kinase (aPK) superfamily. In a further embodiment the component B is a human kinase or a non-human kinase. A further embodiment of the invention is a complex wherein the ePK is selected from the group of calcium/calmodulin-regulated (CaM) death-promoting kinases, consisting of death-associated protein kinase (DAP-kinase, DAPk), DAP kinase-related protein kinase 1 (DRP-1), also named DAP-kinase 2 (DAPk2), DAP like kinase/Zipper interacting protein kinase (Dlk/ZIP-kinase), also named DAP-kinase 3 (DAPK3) and DAP kinase related apoptosis-inducing kinase (DRAK1 and DRAK2) families, the group of Group of calcium/calmodulin-regulated (CaM) death-promoting kinases-like (CAMKL) family, consisting of at least 49 subfamilies, protein kinase AMP-activated alpha-1 catalytic subunit (PRKAA1), protein kinase AMP-activated alpha 2 catalytic subunit (PRKAA2), BRSK1 and BRSK2, CHK1 checkpoint homologue (CHEK1), hormonally upregulated Neu-associated kinase (HUNK), serine/threonine kinase 11 (Peutz-Jeghers syndrome) (STK11), MAP/microtubule affinity-regulating kinase (MARK) 1-4, MARKps 01-30, likely ortholog of maternal embryonic leucine zipper kinase (KIAA0175), PAS domain containing serine/threonine kinase (PASK), NIM1, QIK and SNRK, the group of death-domain receptor interacting protein kinase (RIP-kinase) family, consisting of at least six subfamilies, RIP-kinase 1, RIP-kinase 2, RIP-kinase 3 and RIP-kinase 4, ankyrin repeat domain 3 (ANKRD3)

and SqK288, the group of multifunctional CaM kinase family, consisting of CaM kinases I, II, including the microtubule affinity-regulating kinases (MARK) and microtubule affinity-regulating kinases-like 1 (MARKL1), CaM kinase IV and CaM kinase kinase subfamilies, the group of dedicated CaM kinases, consisting of Myosin light chain kinase (MLCk), phosphorylase kinase and CaM kinase III (eEF-2k), the group of mitogen-activated protein kinase (MAPK) family, consisting of extracellular signal-regulated kinases (ERK), c-JUN NH2-terminal protein kinases (JNK), nemo-like kinase (NLK) and p38 kinase subfamilies, the group of cyclin-dependent kinase (CDK) family, consisting of the subfamilies, cell cycle related kinase (CCRK), cell division cycle 2 (CDC2), cyclin-dependent kinases (CDK) 1-11, PCTAIRE protein kinase (PCTK) 1-3, PFTAIRE protein kinase (PFTK) 1-2 and cell division cycle 2-like 1 (PITSLRE proteins), the group of eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) family, also named (PEK), consisting of the protein kinase interferon-inducible double stranded RNA (dsRNA) dependent (PKR) subfamily. A further embodiment of the present invention concerns a complex wherein the histidine protein kinase is selected from one of the eleven families HPK 1-11. A further embodiment of the present invention is a complex wherein the aPK is selected from the alpha protein kinase family, consisting of eukaryotic elongation factor-2 kinase (eEF-2k), myosin heavy chain kinase (MHC-kinase), eukaryotic translation initiation factor 2 alpha kinase 1 (E2K1) and channel kinase (Chak1 and Chak2) subfamilies, the group of Fas-activated s/t kinase (FASTK) family, consisting of the FASTK subfamily, the group of protein tyrosine kinase 9 (A6) family, consisting of A6 and protein tyrosine kinase 9-like (A6r) subfamilies, the group of p21-activated protein kinases (PAK) family, consisting of the three highly conserved isoforms: alpha-PAK (PAK1), beta-PAK (PAK3) and gamma-PAK (PAK2, PAKI), the group of Interleukin-1 (IL-1)-receptor-associated kinase (IRAK) family, consisting of IRAK-1, IRAK-2, IRAK-3 and IRAK-4 subfamilies, or derivatives, mutants or combinations thereof. A further embodiment is a complex wherein component B directly activates or inactivates components of cell-regulatory pathways, altering the function, gene expression, or viability of a target cell, whereby a target cell is defined by the ability of component A to

bind to the cell. In a further embodiment, component B of the complex is DAPK2 or a derivative thereof or EF-2K or a derivative thereof.

A further embodiment of the present invention is a complex comprising one or more supplementary components S which regulate protein biosynthesis on the transcription and/or translation level, and/or enable purification and/or detection of the complex or its components, and/or facilitate translocation of at least component B into the target cell and intracellular separation therein, and/or activation of component B. A further embodiment of the present invention is a complex wherein the components are chemically coupled and/or genetically fused to each other. A further embodiment are the genetically fused complexes named *L-DAPk2-Ki-4-III/G* (SEQ ID NO: 2), *Ki-4-DAPk2-II/G* (SEQ ID NO: 4) and KI-4(scFv)-eEF-2K (SEQ ID NO: 6), encoded by the corresponding DNA molecules with SEQ ID NOs 1, 3, and 5, respectively. A further embodiment of the present invention are a nucleic acid molecule coding for said complex or for individual components thereof for the preparation of such complex, and/or a vector comprising said nucleic acid molecule. The present invention also concerns cells and non-human organisms synthesizing complete complexes or individual components thereof after having been transformed or transfected with nucleic acid molecules coding for said complexes of the present invention, or *in vitro* translation systems synthesizing complete complexes or individual components thereof. A further embodiment are also an organism and/or a cell transformed or transfected with the nucleic acid molecule or vector encoding said complex or components thereof, whereby said organism is either a prokaryote, such as *E. coli*, *B. subtilis*, *S. carnosus*, *S. coelicolor*, and/or *Marinococcus sp.*, or a lower eukaryote, such as *Saccharomyces sp.*, *Aspergillus sp.*, *Spodoptera sp.* and/or *P. pastoris*, or a higher non-human eukaryote such as a plant and/or an animal, and the cell is a primary or cultivated mammalian cell, such as a freshly isolated human cell or a eukaryotic cell line, such as CHO, Cos or 293. A further embodiment is a method for influencing the growth and/or the physiology of the cells transfected or transformed with the nucleic acid molecule or the vector encoding said complex, by culturing the cells under conditions supporting the activity of the complex. A further embodiment of the

present invention is a kit comprising the complex and/or the nucleic acid molecule and/or the vector, and/or the cells and/or prokaryotes and/or lower eukaryotes transfected or transformed with said nucleic acid molecules of the present invention. A further embodiment is the use of the complex, and/or the nucleic acid molecules, and/or vectors, and/or the cells and/or prokaryotes and/or lower eukaryotes transfected or transformed with said nucleic acid molecules and/or the kit for the preparation of a medicament for the treatment of proliferative diseases, such as cancerous or non-cancerous proliferative diseases, allergies, autoimmune diseases and/or chronic inflammation.

A further embodiment is a medicament comprising a complex, and/or nucleic acid molecules and/or vectors and/or or cells or organisms synthesising the complex of present invention, for treating proliferative diseases, such as cancerous or non-cancerous proliferative diseases, allergies, autoimmune reactions, chronic inflammation reactions or tissue rejection reactions. A further embodiment is the *ex vivo*, *in vivo or in vitro* use of the complex, and/or the nucleic acid molecule and/or the vector, and/or the cells and/or the organisms synthesising the complex and/or the kit, for the targeted modulation of cellular signaling pathways. A further embodiment is the use of the complex, and/or the nucleic acid molecule and/or the vector, and/or the cells and/or organisms synthesising the complex and/or the kit for prognostic, diagnostic, and/or analytic kinase assays, and/or for the the development of such assays. A further embodiment is a method of treatment of proliferative diseases, such as cancerous or non-cancerous proliferative diseases, allergies, autoimmune diseases, and/or chronic inflammation comprising the steps of administering to a patient the complex of the present invention and/or the nucleic acid and/or the vector encoding said complex.

Brief description of the drawings

Figure 1: Cloning of pMS-(L-DAPk2-KI-4)-III/G (SEQ ID NO 1), pMS-(Ki-4-DAPk2)-II/G (SEQ ID NO 3) and pMT-Ki-4(scFv)-eEF-2K (SEQ ID NO 5). Lane 1-3, PCR-amplification of DAPk2 and derivatives thereof. Lane 4, PCR-

amplification of eEF-2K and derivatives thereof. (M, DNA-ladder; C, negative control).

Figure 2: Schematic structure of the eukaryotic expression cassettes pMS-(L-DAPKk2-Ki-4)-III/G (SEQ ID NO 1), pMS-(Ki-4-DAPk2)-II/G (SEQ ID NO 3) and prokaryotic expression module pMT-KI-4(scFv)-eEF-2K coding region. Legends: hCMV = human Cyto-megalovirus promotor/enhan-cer; Ig-*k*-L = Immunoglobin *k*appa-chain leader sequence; M / H = c-Myc epitope (EQKLISEEDL (SEQ ID NO: 8)) and *hexa*-Histidine tag; IVS / IRES = Intervening sequence / internal ribosome entry site; EGFP = enhanced green fluorescent protein; T7-lac = bacteriophage T7 promotor-lactose operator; pelB = bacterial leader/signal sequence pectate lyase B from *Erwinia carotovora* EC; His_{10} = *deca*-Histidine tag; V_H = Immunoglobulin variable heavy-chain; V_L = Immunoglobulin variable light-chain; $(G_4S)_3$ = (Glycine x 4 - serine) x 3 linker; ATG = Translation initiation codon; Stop = Translation termination codon; DAPK2 = Death-associated protein-kinase 2 / DRP-1; eEF-2K = eukaryotic elongation factor-2 kinase; Ki-4 = anti-CD30 immunoglobulin single-chain variable fragment (scFv).

Figure 3: Binding properties of the recombinant anti-CD30 immunokinases. Binding of pMS-(L-DAPk2-Ki-4)-III/G (SEQ ID NO 2) to CD30-positive cells by flow cytometry. Cells were stained with purified Immunokinase (B) or with PBS as negative control (A). Figure 4: Growth inhibition of Hodgkin-derived CD30-positive cell lines after incubation with pMS-(L-DAPk2-Ki-4)-III/G as documented by cell-viability assays. L-540Cy cells were treated with different dilutions of recombinant ani-CD30 immunkinase, and their ability to metabolize the XTT to a water-soluble formazan salt was measured as absorbance at 450 and 650 nm. Measurements were performed in triplicate. Results are presented as percentage of untreated control cells and to Zeocin-treated positive control.

Detailed description of the invention

The complex according to the invention is a recombinant heterologous complex comprising at least two domains, i.e. one effector domain and at least one cell-specific binding domain. The complex according to the invention is usable for diagnosis and therapy of diseases.

The invention described herein draws on previously published work and pending patent applications. By way of example, such work consists of scientific papers, patents or pending patent applications. All of these publications and applications, cited previously or below are hereby incorporated by reference.

Definitions

As used herein, the term "immunotoxin" refers to chimeric molecules in which a cell-binding monoclonal antibody or fragments thereof are chemically coupled or genetically fused to toxins or their subunits. The toxin portion of the immunotoxin can be derived form various sources, such as plants, animals, higher and lower microorganisms such as bacteria and fungi, and in particular if the toxin is a catalytic enzyme, the enzyme can be of human origin. The toxin can also be a synthetic drug. Immunotoxins as well their constructions are reviewed above and are well known to the person skilled in the art.

As used herein, the term "immunokinase" refers to chimeric molecules in which a cell-binding monoclonal antibody or fragments thereof are coupled or fused to kinases or their subunits. The term immunokinase is a synonym for the complex of the present invention.

As used herein, the term "component A" of the complex represents the actively binding structure of the complex of present invention. The component A is selected from the group of actively binding structures consisting of antibodies or their derivatives or fragments thereof, synthetic peptides such as scFv, mimotopes, etc. or chemical molecules such as carbohydrates, lipids, nucleic acids, peptides, vitamins, etc., and/or small molecules with up to 100 atoms with receptor-binding activity like ligands, in particular single atoms,

peptidic molecules, non-peptidic molecules, etc., and/or cell surface carbohydrate binding proteins and their ligands such as lectins, in particular calnexins, c-type lectins, l-type lectins, m-type lectins, p-type lectins, r-type lectins, galectins and their derivatives, and/or receptor binding molecules such as natural ligands to the cluster of differentiation (CD) antigens, like CD30, CD40, etc., cytokines such as chemokines, colony stimulating factors, type-1 cytokines, type-2 cytokines, interferons, interleukins, lymphokines, monokines, etc., and/or adhesion molecules including their derivatives and mutants, and/or derivatives or combinations of any of the above listed of actively binding structures, which bind to CD antigens, cytokine receptors, hormone receptors, growth factor receptors, ion pumps, channel-forming proteins. The component A may also be selected from the group of passively binding structures consisting of allergens, peptidic allergens, recombinant allergens, allergen-idiotypical antibodies, autoimmune-provoking structures, tissue-rejection-inducing structures, immunoglobulin constant regions and their derivatives, mutants or combinations thereof. A component A with higher valency may be generated by combining at least two identical or different binding structures selected from the above mentioned groups.

As used herein, the term "antibody" refers to polyclonal antibodies, monoclonal antibodies, humanized antibodies, single-chain antibodies, and fragments thereof such as Fab, F(ab')2, Fv, and other fragments which retain the antigen binding function and specificity of the parent antibody.

As used herein, the term "monoclonal antibody" refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. The term encompasses whole immunoglobulins as well as fragments such as Fab, F(ab')2, Fv, and others which retain the antigen binding function and specificity of the antibody. Monoclonal antibodies of any mammalian species can be used in this invention. In practice, however, the antibodies will typically be of rat or murine origin because of the availability of rat or murine cell lines for use in making the required hybrid cell lines or hybridomas to produce monoclonal antibodies.

As used herein, the term "human antibodies" means that the framework regions of an immunoglobulin are derived from human immunoglobulin sequences.

As used herein, the term "single chain antibody fragments" (scFv) refers to antibodies prepared by determining the binding domains (both heavy and light chains) of a binding antibody, and supplying a linking moiety, which permits preservation of the binding function. This forms, in essence, a radically abbreviated antibody, having only that part of the variable domain necessary for binding to the antigen. Determination and construction of single chain antibodies are described in U.S. Pat. No. 4,946,778 to Ladner et al.

The "component B" of present invention represents the "targeted kinase" moiety of the immunokinase of the present invention and may be selected from any kinase known in the art. Preferably component B is chosen from the following three classes of kinases: 1. The eukaryotic protein kinase (ePK) superfamily, 2. the histidine protein kinase (HPK) superfamily, or 3. the atypical protein kinase (aPK) superfamily. If component B is chosen from the ePK superfamily, it is selected from the group of calcium/calmodulin-regulated (CaM) death-promoting kinases, consisting of death-associated protein kinase (DAP-kinase, DAPk), DAP kinase-related protein kinase 1 (DRP-1), also named DAP-kinase 2 (DAPk2), DAP like kinase/Zipper interacting protein kinase (Dlk/ZIP-kinase), also named DAP-kinase 3 (DAPK3) and DAP kinase related apoptosis-inducing kinase (DRAK1 and DRAK2) families, the group of calcium/calmodulin-regulated (CaM) death-promoting kinases-like (CAMKL) family, consisting of at least 49 subfamilies, protein kinase AMP-activated alpha 1 catalytic subunit (PRKAA1), protein kinase AMP-activated alpha 2 catalytic subunit (PRKAA2), BRSK1 and BRSK2, CHK1 checkpoint homologue (CHEK1), hormonally upregulated Neu-associated kinase (HUNK), serine/threonine kinase 11 (Peutz-Jeghers syndrome) (STK11), MAP/microtubule affinity-regulating kinase (MARK) 1-4, MARKps 01-30, likely ortholog of maternal embryonic leucine zipper kinase (KIAA0175), PAS domain containing serine/threonine kinase (PASK), NIM1, QIK and SNRK, the group of death-domain receptor interacting protein kinase (RIP-kinase) family, consisting of at least six subfamilies, RIP-kinase 1, RIP-kinase 2, RIP-kinase 3

and RIP-kinase 4, ankyrin repeat domain 3 (ANKRD3) and SqK288, the group of multifunctional CaM kinase family, consisting of CaM kinases I, II, including the microtubule affinity-regulating kinases (MARK) and microtubule affinity-regulating kinases-like 1 (MARKL1), CaM kinase IV and CaM kinase kinase subfamilies, the group of dedicated CaM kinases, consisting of Myosin light chain kinase (MLCk), phosphorylase kinase and CaM kinase III (eEF-2k), the group of mitogen-activated protein kinase (MAPK) family, consisting of extracellular signal-regulated kinases (ERK), c-JUN NH2-terminal protein kinases (JNK), nemo-like kinase (NLK) and p38 kinase subfamilies, the group of cyclin-dependent kinase (CDK) family, consisting of the subfamilies, cell cycle related kinase (CCRK), cell division cycle 2 (CDC2), cyclin-dependent kinases (CDK) 1-11, PCTAIRE protein kinase (PCTK) 1-3, PFTAIRE protein kinase (PFTK) 1-2 and cell division cycle 2-like 1 (PITSLRE proteins), the group of eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) family, also named (PEK), consisting of the protein kinase interferon-inducible double stranded RNA (dsRNA) dependent (PKR) subfamily.

If component B is chosen from the HPK superfamily, it is selected from the group of at least eleven families HPK 1-11.

If component B is chosen from the aPK superfamily, it is selected from the group of alpha protein kinase family, consisting of eukaryotic elongation factor-2 kinase (eEF-2k), myosin heavy chain kinase (MHC-kinase), eukaryotic translation initiation factor 2 alpha kinase 1 (E2K1) and channel kinase (Chak1 and Chak2) subfamilies, the group of Fas-activated s/t kinase (FASTK) family, consisting of the FASTK subfamily, the group of protein tyrosine kinase 9 (A6) family, consisting of A6 and protein tyrosine kinase 9-like (A6r) subfamilies, the group of p21-activated protein kinases (PAK) family, consisting of the three highly conserved isoforms: alpha-PAK (PAK1), beta-PAK (PAK3) and gamma-PAK (PAK2, PAKI), the group of Interleukin-1 (IL-1)-receptor-associated kinase (IRAK) family, consisting of IRAK-1, IRAK-2, IRAK-3 and IRAK-4 subfamilies.

The term "recombinant" refers to the preparation of molecules, in particular the covalent joining of molecules from different sources, by any one of the known methods of molecular biology. As used in the present invention, the

term "recombinant" refers in particular to the fusion of the antibody part to the toxin part by any one of the known methods of molecular biology, such as through production of single chain antibodies. The recombinant DNA molecule encoding the recombinant fusion protein comprising the antibody part and the toxin part are recombinantly expressed. Recombinant immunotoxin produced in this way may be isolated by any technique known in the field of recombinant DNA expression technology suitable for this purpose.

As used herein, the term "vector" comprises DNA and RNA forms of a plasmid, a cosmid, a phage, phagemid, derivatives of them, or a virus. A vector comprises control sequences and coding sequences.

The term "expression of the recombinant genes encoding the recombinant complex", wherein the recombinant complex is a single chain antibody-toxin moiety fusion polypeptide, also called recombinant immunokinase, refers to the transformation and/or transfection of a host cell with a nucleic acid or vector encoding such a complex, and culturing said host cells selected from the group of bacteria, such as *E. coli, and/*or in yeast, such as in *S. cerevisiae*, and/or in established mammalian or insect cell lines, such as CHO, COS, BHK, 293T and MDCK cells, and/or in primary cells, such as human cells, non-human vertebrate cells, and/or in invertebrate cells such as insect cells, and the synthesis and translation of the corresponding mRNA, finally giving rise to the recombinant protein, the recombinant complex. In more detail, the term "expression of the recombinant genes encoding the recombinant complex", comprises the following steps:

Transformation of an appropriate cellular host with a recombinant vector, in which a nucleotide sequence coding for the fusion protein had been inserted under the control of the appropriate regulatory elements, particularly a promoter recognized by the polymerases of the cellular host. In the case of a prokaryotic host, an appropriate ribosome binding site (RBS) also precedes the nucleotide sequence coding for the fusion protein, enabling the translation in said cellular host. In the case of an eukaryotic host any artificial signal sequence or pre/pro sequence may be provided, or the natural signal sequence may be employed. The transformed cellular host is cultured under conditions enabling the expression of said insert.

As used herein, the expression "killing of antigen-expressing cells" refers to the inhibition of protein synthesis or induction of apoptosis, resulting in elimination or death of these cells.

The term "supplementary components S", refers to an additional component of the complex comprising A and B. The supplementary component S contributes features and properties to the complex which allow efficient preparation and/or modify the effectiveness of the complex:

- the inducible regulation of transcription/translation (e.g., inducible promoters);
- control of protein biosynthesis (e.g., leader sequences);
- purification/detection of the complex or its components (e.g., His tag, affinity tags);
- translocation of the apoptotic agents into the target cells (e.g., translocation domain, amphiphatic sequences);
- intracellular activation/separation of component B (synthetic pro-granzyme B, amphiphatic sequences).

The invention also relates to nucleic acid molecules, such as DNA and/or RNA, or vectors, which code for the complex of the present invention or for individual components for preparing the complex. The feasability of the expression of the nucleic acids encoding a recombinant complex in eukaryotic cells of human origin is successfully documented here, as well as the feasibility to use the complex as an specific apoptotic agents in eukaryotic cells of human origin. This suggests the suitability of nucleic acids coding for a complex according to the invention also for non germ line gene-therapeutic approaches. A person skilled in the art is capable of recognizing the various aspects and possibilities of gene-therapeutic interventions in connection with the various diseases to be treated. In addition to the local application of relatively non-specific vectors (e.g., cationic lipids, non-viral, adenoviral and retroviral vectors), a systemic application with modified target-cell-specific vectors will also become possible in the near future. Complexes and nucleic acid molecules and/or vectors coding for the complexes of present invention, are used for the preparation of medicaments for non-germ line gene

therapeutic interventions, for the local or systemic application. An interesting alternative to systemic application are the well-aimed *ex vivo* transfection of defined cell populations and their return into the organism, or the use of the *ex vivo* transfected defined cell populations for the preparation of a medicament for the treatment of diseases associated with these cell populations.

Also claimed are cells or *in vitro* translation systems, which synthesize complete complexes according to the invention or individual components thereof, after transformation and/or transfection with, or addition of the nucleic acid molecules or vectors according to the invention.

Cells or organisms according to the invention are either of prokaryotic origin, especially from *E. coli*, *B. subtilis*, *S. carnosus*, *S. coelicolor*, *Marinococcus sp.*, or eukaryotic origin, especially from *Saccharomyces sp.*, *Aspergillus sp.*, *Spodoptera sp.*, *P. pastoris*, primary or cultivated mammalian cells, eukaryotic cell lines (e.g., CHO, Cos or 293) or plants (e.g. *N. tabacum*).

The invention also relates to medicaments comprising the complex according to the present invention and/or the nucleic acid or vectors encoding the complex of present invention. Typically, the complexes according to the invention are administered in physiologically acceptable dosage forms. These include, for example, Tris, NaCl, phosphate buffers and all approved buffer systems, especially including buffer systems, which are characterized by the addition of approved protein stabilizers. The administration is effected, in particular, by parenteral, intravenous, subcutaneous, intramuscular, intratumoral, transnasal administrations, and by transmucosal application.

The dosage of the complexes according to the invention to be administered must be established for each application in each disease to be newly treated by clinical phase I studies (dose-escalation studies).

Nucleic acids or vectors, which code for a complex according to the invention, are advantageously administered in physiologically acceptable dosage forms. These include, for example, Tris, NaCl, phosphate buffers and all approved buffer systems, especially including buffer systems, which are characterized by the addition of approved stabilizers for the nucleic acids and/or vectors to be used. The administration is effected, in particular, by parenteral, intravenous,

subcutaneous, intramuscular, intratumoral, transnasal administrations, and by transmucosal application.

The complex according to the invention, nucleic acid molecules coding therefore and/or cells or *in vitro* translation systems can be used for the preparation of a medicament for treating tumor diseases, allergies, autoimmune diseases, and chronic/acute inflammation reactions.

Results

Following the construction of three types of recombinant complexes (immunokinases), first results obtained demonstrate their superior quality with regard to binding specificity as well as cytoxicity.

Construction and expression of a recombinant complex (immunokinase)

PCR-amplified DAPK2' DNA (Fig. 1) was directionally cloned into the ampicillin-resistant pMS-(L-ANG-Ki-4)-III/G eukaryotic expression vector containing a *Igk*-leader (L) sequence at the N-terminus, Ki-4(scFv) (component A) and a tandem Myc- and His-Tag epitope at the C-terminus of the expression cassette (Fig. 2) Successful cloning was verified by DNA sequence analysis. Three days after transfection of 293T-cells, the appropriate sized expected recombinant complex (immuno-kinase) pMS-(L-DAPk2-Ki-4)-III/G (M_r ~66,000) was detected by Western blot analysis of protein mini-preparations. Transfected producer-cells were further cultivated under Zeocin selection pressure in medium culture flasks and were used for larger scale production of the recombinant complex (immunokinase) pMS-(L-DAPk2-Ki-4)-III/G. Under normal culture conditions, between 0.1 and 0.5 µg of the recombinant protein were purified from 1 ml cell culture supernatant by a one step Ni-NTA purification procedure. The intact recombinant complex (immunokinase) was secreted into the supernatant of transfected 293T-cells, as visualized by immunoblot using mouse-anti-penta-His monoclonal antibody.

positive cells with a calculated median IC_{50} of betwenn 4 and 35 ng/ml on L540Cy cells (Fig. 4) The CD30-negative Ramos and 8701-BC cell lines were not affected by recombinant immunokinase concentrations of up to 10 µg/ml. Thus the component A (anti-CD30 scFv) of the complex conferred specificity to the recombinant complex, limiting the cytotoxic effects of the kinase domain to the selected target cells.

Examples

Bacterial strains, oligonucleotides, and plasmids

E.coli XL1-blue (supE44 hsdR17 recA1 endA1 gyr A46 thi relA1 lacF'[pro AB$^+$ lacI^q lacZ ΔM15 Tn10(tet^r)]) were used for the propagation of plasmids, and *E.coli* BL21 Star™ (DE3) (F$^-$ ompT hsdS$_B$(r$_B^-$m$_B^-$) gal dcm rne131 DE3) as host for synthesis of recombinant immunokinases. Synthetic oligonucleotides were synthesized by MWG Biotech (Ebersberg, Germany). The bacterial expression vector pBM-KI-4 is derived from the pET27b plasmid (Novagen, Madison, USA), and is used for the expression of the C-terminal fusion of Not I/Bip I-kinase domains to the anti-CD30 scFv (Klimka, A. *et al.*, 1999). The eukaryotic expression vectors pMSKAngII and pMSLAngKIII are derived from the pSecTag plasmid (Invitrogen, Carlsbad, USA) and are used for N- or C-terminal fusion of XbaI/BlpI-kinase domains to the KI-4(scFv) (Stöcker, M. *et al.*, 2003). Plasmids were prepared by the alkaline lysis method and purified using plasmid preparation kits from Qiagen (Hilden, Germany). Restriction fragments or PCR products were separated by horizontal agarose gel electrophoresis and extracted with QIAquick (Qiagen). All standard cloning procedures were carried out as described by Sambrook, J. et al., 1989.

Cell culture

All cell lines, including the CD30-positive cell lines L540Cy (Kapp, U. et al., 1992) and HL-60 (Thepen, T. Utrecht, The Netherlands) the CD30-negative cell lines Ramos (ATCC, VA, USA) and 8701-BC (Minafra, S. et al., 1989) and the producer cell line 293T (ATCC) were cultivated in complex medium (RPMI

PCR-amplified eEF-2K DNA encoding component B (Fig. 1, 4a-e) was directionally cloned into the pET-derived kanamycin-resistant pBM-Ki-4(scFv) prokaryotic expression vector containing an IPTG-inducible *lac* operator, a *pelB* signal peptide followed by an enterokinase-cleavable His_{10} tag, and Ki-4(scFv) (componnet A) (Fig. 2). Successful cloning of the recombinant complex construct pMT-Ki-4(scFv)-eEF-2K was verified by DNA sequence analysis.

After transformation, recombinant *E.coli* BL21 Star™ (DE3) clones were cultivated under osmotic stress conditions in the presence of compatible solutes. The recombinant complex (immunokinase) was directed into the periplasmic space and the functional pMT-Ki-4(scFv)-eEF-2K (M_r ~113,000) protein directly purified by combination of IMAC and SEC to >90% purity. At least 1 mg of purified pMT-Ki-4(scFv)-eEF-2K protein was routinely prepared from 1 liter of bacterial shaking cultures. The intact recombinant complex (immunokinase) was secreted to the periplasmic compartment, as visualized by immunoblot using mouse-anti-penta-His monoclonal antibody.

Binding properties of recombinant complexes (immunokinases)

Fusing the Ki-4(scFv) coding regions, component A of the complex, to the kinase coding sequences, component B of the complex, did not affect the binding activity of the V_H/V_L antibody format of component A. Component A conferred specificity against the CD30 molecule. The purified recombinant complex (immunokinase) comprising the anti-CD30 component A always bound to the Hodgkin-derived cell line L540Cy as measured by flow cytometry (Fig. 3).

In vitro cytotoxic activity

To characterize the cytotoxic activity of the recombinant complex comprising anti-CD30 (as component A) and kinases (component B) *in vitro*, the proliferation of different target cells was evaluated after incubation with different amounts of the recombinant complexes (immunokinases) pMS-(L-DAPk2-Ki-4)-III/G and pMT-Ki-4(scFv)-eEF-2K, respectively. Growth inhibition of the CD30-positive cell lines L540Cy and HL60 were documented by a XTT-based colorimetric assay. Toxic effects were observed only against CD30-

1640) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 50 µg/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. All cells were cultured at 37°C in a 5% CO_2 in air atmosphere. For the selection of transfected cells, Zeocin (Invitrogen) was added to a final concentration of 100 µg/ml.

Construction and expression of recombinant complexes (immunokinases)

Cloning and expression of pMS-(L-DAPk2-Ki-4)-III/G (SEQ ID NO 1) and pMS-(KI-4-DAPk2)-II/G (SEQ ID NO 3)

For the construction of a vector encoding a recombinant complex with N- or C-terminal DAP-kinase 2 (DAPk2)-fusions, DAPk2 was PCR amplified to introduce the restriction sites *XbaI* and *BlpI*. After *XbaI/BlpI*-digestion, the PCR-product was cloned into the eukaryotic expression vector pMS-(L-ANG-Ki-4)-III/G and pMS-(Ki-4-ANG)-II/G respectively, digested with the same restriction enzymes. The resulting recombinant constructs pMS-(L-DAPk2-Ki-4)-III/G (SEQ ID NO: 1) and pMS-(KI-4-DAPk2)-II/G (SEQ ID NO: 3) encoding the immukinase proteins L-DAPk2-Ki-4-MH (SEQ ID NO 2) and L-Ki-4-DAPk2-MH (SEQ ID NO 4) were verified by sequence analysis. After *TransFast*-mediated (Promega, Mannhein, Germany) transformation into 293T-cells, the recombinant immunokinase was expressed as described by Stöcker M. *et al.*, 2003. Briefly, one µg plasmid-DNA and 3µl TransFast have been used according to the manufactures protocol for 12 well cell culture plates. Transfection efficiency was between 75 and 95% determined by counting green fluorescent cells. 3 days after initial transfection, cell culture supernatants were analyzed for recombinant protein. Subsequently, transfected cells were transferred into medium-sized cell culture flasks (Nunc; 85m^2) and grown in RPMI complex medium supplemented with 100 µg/ml Zeocin. One to two weeks productively transfected clones were green fluorescing and hence could be detected by fluorescence microscopy. Transfected cell populations were established by subcultivation of these clones. Purifications of the His-tagged proteins were accomplished by the Ni-NTA metal-affinity method (Hochuli, V., 1989, Porath, J. *et al.*, 1975) (Qiagen). The protein purification followed a modified protocol for the

purification of native protein from Qiagen (*The Expressionist* 07/97). For protein mini-preparation, 900 µl centrifugation-cleared cell culture supernatant was supplemented with 300µl of 4x incubation buffer (200mM NaH_2PO_4, pH 8.0; 1.2M NaCl; 40mM Imidazol) and 30µl 50% Ni-NTA. Following 1h incubation, the Ni-NTA resin was pelleted by centrifugation. After washing the sediment twice in 175 µl 1x incubation buffer, bound protein was eluted with 30 µl of elution buffer (50mM NaH_2PO_4, pH 8.0; 1.2M NaCl; and 40 mM imidazol) and 30µl 50% Ni-NTA. Following an 1 h incubation, the Ni-NTA resin was pelleted by centrifugation. After washing the sediment twice in 175 µl 1x incubation buffer, bound protein was eluted with 30 µl of elution buffer (50mM NaH_2PO_4, pH8.0; 300mM NaCl; 250mM Imidazol) for 20min at RT. Larger scale purification of eukaryotically-expressed proteins up to 500ml cell culture supernatant was performed on a BioLogic workstation (Bio-Rad, USA). Cell culture supernatants were loaded onto a Ni-NTA column and following elution of the His-tagged proteins were made under the conditions described above.

Cloning and expression of pMT-Ki-4(scFv)-eEF-2K

The eukaryotic elongation factor-2 kinase (eEF-2k) was amplified by PCR to introduce the restriction sites *NotI* and *BlpI*. After *NotI/BlpI*-digestion, the PCR-fragment was cloned into the bacterial expression vector pBM-Ki-4, digested with the same restriction enzymes. The resulting recombinant construct pMT-Ki-4(scFv)-eEF-2K (SEQ ID NO: 5) was verified by DNA sequence analysis. After transformation into BL21 Star™ (DE3), the immunokinase Ki-4(scFv)-eEF-2K (SEQ ID NO 6) were periplasmically expressed under osmotic stress in the presence of compatible solutes as described by Barth, S. *et al.* 2000. Briefly, transformed bacteria were harvested 15 h after IPTG induction. The bacterial pellet was resuspended in sonication-buffer (75 mM Tris/HCl (pH 8), 300 mM NaCl, 1 capsule of protease inhibitors/ 50 ml (Complete™, Roche Diagnostics, Mannheim, Germany), 5 mM DTT, 10 mM EDTA, 10% (v/v) glycerol) at 4°C and sonicated 6 times for 30 s at 200 W. The m22(scFv)-ETA' fusion proteins were enriched by IMAC (immobilized metal-ion affinity chromatography) using nickel-nitriloacetic chelating Sepharose (Qiagen) and SEC (size exclusion chromatography) with

Bio-Prep SE-100/17 (Biorad, München, Germany) columns according to the manufacturer's instructions. Recombinant Protein was eluted with PBS (pH 7.4) and 1 M NaCl, analyzed by Sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS-PAGE), quantified by densitometry (GS-700 Imaging Densitometer; Biorad) after Coomassie staining in comparison with BSA standards and verified by Bradford assays (Biorad).

SDS-PAGE and Western Blot Analysis

SDS-PAGE, Coomassie staining, and Western blotting were performed as described by Barth, S. *et al.*, 1998. Briefly, recombinant His-tagged immunokinases were detected by mouse-anti-penta-His moab (Qiagen). Bound antibody was detected by a horseradish-conjugated donkey-anti-mouse-IgG moab. (Dianova, Hamburg, Germany), followed by ECL-mediated (Amersham Biosciences, Freiburg, Germany), chemiluminescence reaction and exposition to appropriate X-ray film (Roche, Penzberg, Germany) or alkaline-phosphatase-conjugated anti-mouse-IgG moab (Sigma Chemical Co., Deisenhofen, Germany) and a solution of Tris-HCl (pH 8.0) and 0.2 mg/ml naphtol-AS-Bi-phosphate (Sigma Chemical Co.) supplemented with 1 mg/ml Fast-Red (Serva, Heidelberg, Germany).

Cell membrane (CM) ELISA

The binding activity of recombinant complexes (immunokinases) were determined by CM-ELISA using biological active membranes of tumor cells as described recently by Tur, MK. *et al.*, 2003. Briefly, ELISA Maxisorp-Plates (Nalge Nunc International, Roskilde, Denmark) were coated with 100 µl (~ 0.9 mg protein/ml) freshly prepared membrane fractions of CD30-positive L540Cy/HL60 cells and Ramos/8701-BC as control in 0.02 M bicarbonate buffer, pH 9.6, overnight at 4°C. Plates were washed five times with PBS (pH 7.4) containing 0.2% Tween 20 (TPBS) and blocked with 200 µl 2% BSA in PBS. After overnight incubation at 4°C, plates were washed five times with TPBS and 1 - 10 µg/ml of recombinant immunokinases diluted with 0.5% BSA (w/v) and 0.05% Tween 20 (v/v) in PBS was added to the plates and incubated at RT (23°C) for 1h. Peroxidase labeled anti-His IgG conjugate

(Qiagen) were added diluted with 0.5% BSA and 0.05% Tween 20 in PBS according to manufactures instructions. Bound antibodies were visualized after addition of 100 µl 2', 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) solution (Roche Molecular Biochemical's, Mannheim, Germany) by measuring the extinction at 415 nm with an ELISA-Reader (MWG Biotech).

Flow cytometry analyses

Cell binding activity of the recombinant complexes (immunokinases) expressed in *E.coli* BL21 Star™ (DE3) was evaluated using a FACSCalibur flow cytometry instrument and CellQuest software (Becton Dickinson, Heidelberg, Germany). Cells were stained with recombinant protein as described (25). Briefly, ten thousand events were collected for each sample, and analyses of intact cells were performed using appropriate scatter gates to exclude cellular debris and aggregates. 5 x 10^5 cells were incubated for 1 h on ice with 50 µl of bacterial protein extract at a concentration of 30-40 µg/ml or 100 µl of the immunokinase containing supernatants respectively. The cells were washed with PBS buffer containing 0.2% w/v BSA and 0.05% w/v sodium azide (PBA) and then incubated for 30 min with anti-penta-His moab (Qiagen) diluted 1:2 in PBA buffer. Cells were washed and incubated with fluorescein-isothiocyanate (FITC)-labeled goat-anti-mouse IgG (DAKO Diagnostica, Hamburg, Germany) for 1h at 4°C. After a final wash, the cells were treated with 2µl 6.25 mg/ml propidiumiodide and subsequently analyzed on a FACScalibur (Becton Dickison, Heidelberg, Germany).

Colorimetric cell proliferation assay

The cytotoxic effect of the recombinant complexes (immunokinases) on target cells was determined by measurement of metabolization of yellow tetrazolium salt (XTT) to a water soluble orange formazan dye was determined as published by Barth, S. et al. 2000. Various dilutions of the recombinant immunokinase were distributed in 100 µl-aliquots in 96-well plates. Two-four x 10^4 target cells in 100 µl aliquots of complete medium were added and the plates were incubated for 48 h at 37°C. Afterwards, the cell cultures were pulsed with 100 µl fresh culture medium supplemented with XTT/PMS (final

concentrations of 0.3 mg and 0.383 ng respectively) for 4 h. The spectrophotometrical absorbances of the samples were measured at 450 and 650 nm (reference wavelength) with an ELISA reader (MWG Biotech). The concentration required to achieve a 50% reduction of protein synthesis (IC_{50}) relative to untreated control cells and to 1% Triton X treated positive controls was calculated graphically via Excel generated diagrams. All measurements were done in triplicate.

REFERENCES

1. Kaminski, M. S., Zasadny, K. R., Francis, I. R., Fenner, M. C., Ross, C. W., Milik, A. W., Estes, J., Tuck, M., Regan, D., Fisher, S., Glenn, S. D., and Wahl, R. L. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. J Clin Oncol, *14:* 1974-1981, 1996.

2. Pennell, C. A. and Erickson, H. A. Designing immunotoxins for cancer therapy. Immunol Res, *25:* 177-191, 2002.

3. Chaudhary, V. K., Gallo, M. G., FitzGerald, D. J., and Pastan, I. A recombinant single-chain immunotoxin composed of anti-Tac variable regions and a truncated diphtheria toxin. Proc Natl Acad Sci U S A, *87:* 9491-9494, 1990.

4. Brinkmann, U., Keppler-Hafkemeyer, A., and Hafkemeyer, P. Recombinant immunotoxins for cancer therapy. Expert Opin Biol Ther, *1:* 693-702, 2001.

5. Frankel, A. E., Tagge, E. P., and Willingham, M. C. Clinical trials of targeted toxins. Semin Cancer Biol, *6:* 307-317, 1995.

6. Youle, R. J., Newton, D., Wu, Y. N., Gadina, M., and Rybak, S. M. Cytotoxic ribonucleases and chimeras in cancer therapy. Crit Rev Ther Drug Carrier Syst, *10:* 1-28, 1993.

7. Fett, J. W., Strydom, D. J., Lobb, R. R., Alderman, E. M., Bethune, J. L., Riordan, J. F., and Vallee, B. L. Isolation and characterization of angiogenin, an angiogenic protein from human carcinoma cells. Biochemistry, *24:* 5480-5486, 1985.

8. Huhn, M., Sasse, S., Tur, M. K., Matthey, B., Schinkothe, T., Rybak, S. M., Barth, S., and Engert, A. Human angiogenin fused to human CD30 ligand (Ang-CD30L) exhibits specific cytotoxicity against CD30-positive lymphoma. Cancer Res, *61:* 8737-8742, 2001.

9. Newton, D. L. and Rybak, S. M. Preparation and preclinical characterization of RNase-based immunofusion proteins. Methods Mol Biol, *160:* 387-406, 2001.

10. Goueli, S. Protein Kinases as Drug Targets in High-Throughput Systems. Promega Notes, *75:* 24-28, 2000.

11. Manning, G., Whyte, D. B., Martinez, R., Hunter, T., and Sudarsanam, S. The Protein Kinase Complement of the Human Genome. Science, *298:* 1912-1934, 2002.

12. Hanks, S. K., Quinn, A. M., and Hunter, T. The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. Science, *241:* 42-52, 1988.

13. Ryazanov, A. G., Pavur, K. S., and Dorovkov, M. V. Alpha-kinases: a new class of protein kinases with a novel catalytic domain. Curr Biol, *9:* 43-45, 1999.

14. Braun, A. P. and Schulman, H. The multifunctional calcium/calmodulin-dependent protein kinase: from form to function. Annu Rev Physiol, *57:* 417-445, 1995.

15. Deiss, L. P., Feinstein, E., Berissi, H., Cohen, O., and Kimchi, A. Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev, *9:* 15-30, 1995.

16. Nakatsuka, S., Takakuwa, T., Tomita, Y., Hoshida, Y., Nishiu, M., Yamaguchi, M., Nishii, K., Yang, W. I., and Aozasa, K. Hypermethylation of death-associated protein (DAP) kinase CpG island is frequent not only in B-cell but also in T- and natural killer (NK)/T-cell malignancies. Cancer Sci, *94:* 87-91, 2003.

17. Cohen, O., Feinstein, E., and Kimchi, A. DAP-kinase is a Ca2+/calmodulin-dependent, cytoskeletal-associated protein kinase, with cell death-inducing functions that depend on its catalytic activity. Embo J, *16:* 998-1008, 1997.

18. Pavur, K. S., Petrov, A. N., and Ryazanov, A. G. Mapping the functional domains of elongation factor-2 kinase. Biochemistry, *39:* 12216-12224, 2000.

19. Diggle, T. A., Subkhankulova, T., Lilley, K. S., Shikotra, N., Willis, A. E., and Redpath, N. T. Phosphorylation of elongation factor-2 kinase on serine 499 by cAMP-dependent protein kinase induces Ca2+/calmodulin-independent activity. Biochem J, *353:* 621-626, 2001.

CLAIMS

1. A complex formed from at least one component A and at least one component B, whereby component A comprises a binding domain for cellular surface structures, and component B has kinase properties.

2. The complex according to claim 1, whereby the component A is selected from the group of actively binding structures consisting of antibodies or their derivatives or fragments thereof, and/or synthetic peptides such as scFv, mimotopes, and/or chemical molecules such as carbohydrates, lipids, nucleic acids, peptides, vitamins, and/or small molecules with up to 100 atoms with receptor-binding activity such as ligands, in particular single atoms, peptidic molecules, non-peptidic molecules, and/or cell surface carbohydrate binding proteins and their ligands such as lectins, in particular calnexins, c-type lectins, l-type lectins, m-type lectins, p-type lectins, r-type lectins, galectins and their derivatives, and/or receptor binding molecules such as natural ligands to the cluster of differentiation (CD) antigens, like CD30, CD40, cytokines such as chemokines, colony stimulating factors, type-1 cytokines, type-2 cytokines, interferons, interleukins, lymphokines, monokines, and/or adhesion molecules including their derivatives and mutants, and/or derivatives or combinations of any of the above listed actively binding structures, which bind to CD antigens, cytokine receptors, hormone receptors, growth factor receptors, ion pumps, channel-forming proteins.

3. The complex according to anyone of claims 1 and 2, whereby component A is selected from the group of passively binding structures consisting of allergens, peptidic allergens, recombinant allergens, allergen-idiotypical antibodies, autoimmune-provoking structures, tissue-rejection-inducing structures, immunoglobulin constant regions and their derivatives, mutants or combinations thereof.

4. The complex according to anyone of the claims 1 to 3, wherein the component A directs the complex to a target cell comprising the binding partner for the binding structures of claims 2 and 3.

5. The complex according to anyone the of claims 1 to 4, wherein component A has higher valency by comprising two or more binding structures selected from anyone of those listed in claims 2 and/or 3.

6. The complex according to anyone of the claims 1 to 5, wherein component B is at least one kinase chosen from the following three classes of kinases: 1. eukaryotic protein kinase (ePK) superfamily, 2. histidine protein kinase (HPK) superfamily or 3. atypical protein kinase (aPK) superfamily.

7. The complex according to claim 6, wherein the ePK is selected from the group of calcium/calmodulin-regulated (CaM) death-promoting kinases, consisting of death-associated protein kinase (DAP-kinase, DAPk), DAP kinase-related protein kinase 1 (DRP-1), also named DAP-kinase 2 (DAPk2), DAP like kinase/Zipper interacting protein kinase (Dlk/ZIP-kinase), also named DAP-kinase 3 (DAPK3) and DAP kinase related apoptosis-inducing kinase (DRAK1 and DRAK2) families, the group of Group of calcium/calmodulin-regulated (CaM) death-promoting kinases-like (CAMKL) family, consisting of at least 49 subfamilies, protein kinase AMP-activated alpha 1 catalytic subunit (PRKAA1), protein kinase AMP-activated alpha 2 catalytic subunit (PRKAA2), BRSK1 and BRSK2, CHK1 checkpoint homologue (CHEK1), hormonally upregulated Neu-associated kinase (HUNK), serine/threonine kinase 11 (Peutz-Jeghers syndrome) (STK11), MAP/microtubule affinity-regulating kinase (MARK) 1-4, MARKps 01-30, likely ortholog of maternal embryonic leucine zipper kinase (KIAA0175), PAS domain containing serine/threonine kinase (PASK), NIM1, QIK and SNRK, the group of death-domain receptor interacting protein kinase (RIP-kinase) family, consisting of at least six subfamilies, RIP-kinase 1, RIP-kinase 2, RIP-kinase 3 and RIP-

kinase 4, ankyrin repeat domain 3 (ANKRD3) and SqK288, the group of multifunctional CaM kinase family, consisting of CaM kinases I, II, including the microtubule affinity-regulating kinases (MARK) and microtubule affinity-regulating kinases-like 1 (MARKL1), CaM kinase IV and CaM kinase kinase subfamilies, the group of dedicated CaM kinases, consisting of Myosin light chain kinase (MLCk), phosphorylase kinase and CaM kinase III (eEF-2k), the group of mitogen-activated protein kinase (MAPK) family, consisting of extracellular signal-regulated kinases (ERK), c-JUN NH2-terminal protein kinases (JNK), nemo-like kinase (NLK) and p38 kinase subfamilies, the group of cyclin-dependent kinase (CDK) family, consisting of the subfamilies, cell cycle related kinase (CCRK), cell division cycle 2 (CDC2), cyclin-dependent kinases (CDK) 1-11, PCTAIRE protein kinase (PCTK) 1-3, PFTAIRE protein kinase (PFTK) 1-2 and cell division cycle 2-like 1 (PITSLRE proteins), the group of eukaryotic translation initiation factor 2-alpha kinase 3 (EIF2AK3) family, also named (PEK), consisting of the protein kinase interferon-inducible double stranded RNA (dsRNA) dependent (PKR) subfamily.

8. The complex according to claim 6, wherein the histidine protein kinase is selected from one of the eleven families HPK 1-11.

9. The complex according to claim 6, wherein the aPK is selected from the of alpha protein kinase family, consisting of eukaryotic elongation factor-2 kinase (eEF-2k), myosin heavy chain kinase (MHC-kinase), eukaryotic translation initiation factor 2 alpha kinase 1 (E2K1) and channel kinase (Chak1 and Chak2) subfamilies, the group of Fas-activated s/t kinase (FASTK) family, consisting of the FASTK subfamily, the group of protein tyrosine kinase 9 (A6) family, consisting of A6 and protein tyrosine kinase 9-like (A6r) subfamilies, the group of p21-activated protein kinases (PAK) family, consisting of the three highly conserved isoforms: alpha-PAK (PAK1), beta-PAK (PAK3) and gamma-PAK (PAK2, PAKI), the group of Interleukin-1 (IL-1)-receptor-associated

kinase (IRAK) family, consisting of IRAK-1, IRAK-2, IRAK-3 and IRAK-4 subfamilies, or derivatives, mutants or combinations thereof.

10. The complex according to anyone of the claims 1 to 9, whereby component B directly activates or inactivates components of cell-regulatory pathways, altering the function, gene expression, or viability of a target cell, whereby the target cell is defined by the binding of component A to it.

11. The complex according to anyone of the claims 1 to 10, whereby component B comprises DAPK2 or a derivative thereof.

12. The complex according to anyone of the claims 1 to 10, whereby component B comprises EF-2K or a derivative thereof.

13. The complex according to anyone of the claims 1 to 12, comprising one or more supplementary component S which regulates protein biosynthesis on the transcription and/or translation level, and/or enables purification and/or detection of the complex, and/or facilitates translocation of at least component B into the target cell, and intracellular separation and/or activation of component B.

14. The complex according to anyone of the claims 1 to 13, wherein the components are chemically coupled and/or genetically fused to each other.

15. The complex according to anyone of claims 1 to 14, having the amino acid sequences of SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 6.

16. A nucleic acid molecule coding for the complex according to anyone of claims 1 to 15 or for individual components thereof for the preparation of such complex, and/or a vector comprising said nucleic acid molecule.

17. A cell or non-human organism after having been transformed or transfected with the nucleic acid molecule or vector according to claim 16, and/or an *in vitro* translation systems synthesizing the complete complex according to anyone of the claims 1 to 15 or individual components thereof.

18. The organism or cell according to claim 17, wherby the organism is either a prokaryote, such as *E. coli*, *B. subtilis*, *S. camosus*, *S. coelicolor*, and/or *Marinococcus sp.*, or a lower eukaryote, such as *Saccharomyces sp.*, *Aspergillus sp.*, *Spodoptera sp.* and/or *P. pastoris*, a higher non-human eukaryote such as a plant and/or an animal, and the cell is a primary or cultivated mammalian cell, such as a freshly isolated human cell or a eukaryotic cell line such as CHO, Cos or 293.

19. A method for influencing the growth and/or the physiology of the cells according to anyone of the claims 18 and 19, by culturing the cells under conditions supporting the activity of the complex.

20. A kit comprising the complexes according to anyone of the claims 1 to 15, and/or the nucleic acid molecule and/or the vector of claim 16, and/or the cells and/or non-human organisms of claims 17 or 18.

21. Use of the complex of claims 1 to 15 and/or the nucleic acid molecule and/or vector of claim 16, and/or the cells and/or non-human organisms of claims 17 or 18, and/or the kit of claim 20 for the preparation of a medicament for the treatment of proliferative diseases, such as cancerous or non-cancerous proliferative diseases, allergies, autoimmune diseases, and/or chronic inflammation.

22. A medicament comprising the complex according to anyone of the claims 1 to 15, the nucleic acid molecule and/or vector according to claim 16, or the cells or non-human organisms according to either one of claims 18 or 19.

23. Use of the complex according to anyone of the claims 1 to 15, and/or of the nucleic acid molecules and/or vectors of claim 16, and/or of the cells and/or non-human organisms of claims 17 or 18, and/or the the kit according to claim 20 for targeted modulation of cellular signaling pathways.

24. Use of the complex according to any of the claims 1 to 15, of the nucleic acid molecules and/or vectors of 16, and/or of the cells and/or the non-human organisms of claims 17 or 18, for the development of prognostic, diagnostic, and analytic kinase assays.

ABSTRACT

A complex formed from at least one component A and at least one component B, characterized in that component A has a binding activity for cellular surface structures, and component B is a kinase. The complex allows to influence the growth and the physiology of cells. In particular said complex, nucleic acid molecules encoding it, cells transfected or transformed with these nucleic acid molecules are usable for the preparation of medicaments for the treatment of proliferative diseases, inflammatory diseases, allergies and autoimmune diseases.

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agc ctg acc tct gag gat tct gca gtc tat tac tgt gca aaa aag aca   384
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Lys Lys Thr
        115                 120                 125

act cag act acg tgg ggg ttt cct tct tgg ggc caa ggg acc acg gtc   432
Thr Gln Thr Thr Trp Gly Phe Pro Phe Trp Gly Gln Gly Thr Thr Val
    130                 135                 140

acc gtc tcc tca ggt gga ggc ggt tca ggc gga ggt ggc tct ggc ggt   480
Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
145                 150                 155                 160

ggc gga tcg gac att gtg ctg acc cag tct cca aaa tcc atg gcc atg   528
Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Lys Ser Met Ala Met
                165                 170                 175

tca gtc gga gag agg gtc acc ttg agc tgc aag gcc agt gag aat gtg   576
Ser Val Gly Glu Arg Val Thr Leu Ser Cys Lys Ala Ser Glu Asn Val
            180                 185                 190

gat tct ttt gtt tcc tgg tat caa cag aaa cca ggc cag tct cct aaa   624
Asp Ser Phe Val Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
        195                 200                 205

ctg ctg ata tac ggg gcc tcc aac cgg tac act ggg gtc ccc gat cgc   672
Leu Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
    210                 215                 220

ttc gca ggc agt gga tct gga aga gat ttc act ctg acc atc agc agt   720
Phe Ala Gly Ser Gly Ser Gly Arg Asp Phe Thr Leu Thr Ile Ser Ser
225                 230                 235                 240

gtg cag gct gaa gac ctt gca gat tat cac tgt gga cag aat tac agg   768
Val Gln Ala Glu Asp Leu Ala Asp Tyr His Cys Gly Gln Asn Tyr Arg
                245                 250                 255

tat ccg ctc acg ttc ggt gct ggc acc aag ctg gaa atc aaa cgg gcg   816
Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Ala
            260                 265                 270

gcc gca ctc gag tct aga atg gtc cag gcc tcg atg agg agc cca aat   864
Ala Ala Leu Glu Ser Arg Met Val Gln Ala Ser Met Arg Ser Pro Asn
        275                 280                 285

atg gag acg ttc aaa cag cag aag gtg gag gac ttt tat gat att gga   912
Met Glu Thr Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
    290                 295                 300

gag gag ctg ggc agt ggc cag ttt gcc atc gtg aag aag tgc cgg gag   960
Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
305                 310                 315                 320

aag agc acg ggg ctg gag tat gca gcc aag ttc att aag aag agg cag  1008
Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
                325                 330                 335

agc cgg gcc agc cgt cgg ggc gtg tgc cgg gag gaa atc gag cgg gag  1056
Ser Arg Ala Ser Arg Arg Gly Val Cys Arg Glu Glu Ile Glu Arg Glu
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                340                 345                 350

gtg agc atc ctg cgg cag gtg ctg cac ccc aac atc atc acg ctg cac   1104
Val Ser Ile Leu Arg Gln Val Leu His Pro Asn Ile Ile Thr Leu His
        355                 360                 365

gac ctc tat gag aac cgc acc gac gtg gtg ctc atc ctt gag cta gtg   1152
Asp Leu Tyr Glu Asn Arg Thr Asp Val Val Leu Ile Leu Glu Leu Val
    370                 375                 380

tcc gga gga gaa ctg ttt gat ttc ctg gcc cag aag gag tcg tta agt   1200
Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
385                 390                 395                 400

gag gag gaa gcc acc agc ttc att aag cag atc ctg gat ggg gtg aat   1248
Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn
                405                 410                 415

tac ctt cac aca aag aaa att gct cac ttt gat ctc aag cca gaa aac   1296
Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn
            420                 425                 430

atc atg ttg tta gac aag aat atc cca att cca cac atc aag ctg att   1344
Ile Met Leu Leu Asp Lys Asn Ile Pro Ile Pro His Ile Lys Leu Ile
        435                 440                 445

gac ttt ggc ctg gct cac gaa ata gaa gat gga gtt gaa ttt aaa aac   1392
Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn
    450                 455                 460

att ttt ggg aca cct gaa ttt gtt gct cca gaa atc gtg aac tat gag   1440
Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
465                 470                 475                 480

cca ctg gga ctg gag gcc gac atg tgg agc att gga gtc atc acc tat   1488
Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
                485                 490                 495

atc ctt cta agt gga gcg tcc ccc ttc ctg gga gac aca aaa caa gaa   1536
Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu
            500                 505                 510

acc ctg gca aat atc act gct gtg agt tac gac ttt gat gag gaa ttc   1584
Thr Leu Ala Asn Ile Thr Ala Val Ser Tyr Asp Phe Asp Glu Glu Phe
        515                 520                 525

ttc agc cag aca agc gag ctg gcc aag gac ttc att cgg aag ctt ctt   1632
Phe Ser Gln Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu
    530                 535                 540

gtg aaa gag acc cgg aaa cgg ctt acc atc caa gag gct ctc aga cat   1680
Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His
545                 550                 555                 560

ccc tgg atc gga tcc aaa cta gct gag cac gaa ttt cga gga ggg ccc   1728
Pro Trp Ile Gly Ser Lys Leu Ala Glu His Glu Phe Arg Gly Gly Pro
                565                 570                 575

gaa caa aaa ctc atc tca gaa gag gat ctg aat agc gcc gtc gac cat   1776
Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His
            580                 585                 590

cat cat cat cat cat tga                                         1794
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His His His His His
595

<210> 4
<211> 597
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence:
pMS-(Ki-4-DAPK2')-II/G ORF

<400> 4

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Gly Ser Thr Gly Asp Ala Ala Gln Pro Ala Met Ala Gln Val Lys Leu
            20                  25                  30
Gln Glu Ser Gly Thr Glu Leu Ala Lys Pro Gly Ala Ala Val Lys Met
        35                  40                  45
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr Trp Met His Trp
    50                  55                  60
Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn
65                  70                  75                  80
Pro Asn Thr Ala Tyr Thr Asp Tyr Asn Gln Lys Phe Lys Asp Lys Ala
                85                  90                  95
Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Arg
            100                 105                 110
Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Lys Lys Thr
        115                 120                 125
Thr Gln Thr Thr Trp Gly Phe Pro Phe Trp Gly Gln Gly Thr Thr Val
    130                 135                 140
Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
145                 150                 155                 160
Gly Gly Ser Asp Ile Val Leu Thr Gln Ser Pro Lys Ser Met Ala Met
                165                 170                 175
Ser Val Gly Glu Arg Val Thr Leu Ser Cys Lys Ala Ser Glu Asn Val
            180                 185                 190
Asp Ser Phe Val Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
        195                 200                 205
Leu Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg
    210                 215                 220
Phe Ala Gly Ser Gly Ser Gly Arg Asp Phe Thr Leu Thr Ile Ser Ser
225                 230                 235                 240
Val Gln Ala Glu Asp Leu Ala Asp Tyr His Cys Gly Gln Asn Tyr Arg
                245                 250                 255
Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Ala
            260                 265                 270
Ala Ala Leu Glu Ser Arg Met Val Gln Ala Ser Met Arg Ser Pro Asn
        275                 280                 285
Met Glu Thr Phe Lys Gln Gln Lys Val Glu Asp Phe Tyr Asp Ile Gly
    290                 295                 300
Glu Glu Leu Gly Ser Gly Gln Phe Ala Ile Val Lys Lys Cys Arg Glu
305                 310                 315                 320
Lys Ser Thr Gly Leu Glu Tyr Ala Ala Lys Phe Ile Lys Lys Arg Gln
                325                 330                 335
Ser Arg Ala Ser Arg Arg Gly Val Cys Arg Glu Glu Ile Glu Arg Glu
            340                 345                 350
Val Ser Ile Leu Arg Gln Val Leu His Pro Asn Ile Ile Thr Leu His
        355                 360                 365
Asp Leu Tyr Glu Asn Arg Thr Asp Val Val Leu Ile Leu Glu Leu Val
    370                 375                 380
Ser Gly Gly Glu Leu Phe Asp Phe Leu Ala Gln Lys Glu Ser Leu Ser
385                 390                 395                 400
Glu Glu Glu Ala Thr Ser Phe Ile Lys Gln Ile Leu Asp Gly Val Asn
```

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                    405                 410                 415
Tyr Leu His Thr Lys Lys Ile Ala His Phe Asp Leu Lys Pro Glu Asn
                420                 425                 430
Ile Met Leu Leu Asp Lys Asn Ile Pro Ile Pro His Ile Lys Leu Ile
            435                 440                 445
Asp Phe Gly Leu Ala His Glu Ile Glu Asp Gly Val Glu Phe Lys Asn
        450                 455                 460
Ile Phe Gly Thr Pro Glu Phe Val Ala Pro Glu Ile Val Asn Tyr Glu
465                 470                 475                 480
Pro Leu Gly Leu Glu Ala Asp Met Trp Ser Ile Gly Val Ile Thr Tyr
                485                 490                 495
Ile Leu Leu Ser Gly Ala Ser Pro Phe Leu Gly Asp Thr Lys Gln Glu
            500                 505                 510
Thr Leu Ala Asn Ile Thr Ala Val Ser Tyr Asp Phe Asp Glu Glu Phe
        515                 520                 525
Phe Ser Gln Thr Ser Glu Leu Ala Lys Asp Phe Ile Arg Lys Leu Leu
    530                 535                 540
Val Lys Glu Thr Arg Lys Arg Leu Thr Ile Gln Glu Ala Leu Arg His
545                 550                 555                 560
Pro Trp Ile Gly Ser Lys Leu Ala Glu His Glu Phe Arg Gly Gly Pro
                565                 570                 575
Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Asn Ser Ala Val Asp His
            580                 585                 590
His His His His His His
        595
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<210> 5
<211> 3102
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: pMT-Ki-4 (scFv)-eEF-2K ORF

<220>
<221> CDS
<222> (1)..(3102)

<220>
<221> N region
<222> (2)..(22)
<223> pelB leader sequence

<400> 5

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atg aaa tac ctg ctg ccg acc gct gct gct ggt ctg ctg ctc ctc gct   48
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

gcc cag ccg gcg atg gcc atg ggc cat cat cat cat cat cat cat cat   96
Ala Gln Pro Ala Met Ala Met Gly His His His His His His His His
             20                  25                  30

cat cac agc agc ggc cat atc gac gac gac gac aag cat atg aag ctt  144
His His Ser Ser Gly His Ile Asp Asp Asp Asp Lys His Met Lys Leu
         35                  40                  45

atg gcc cag ccg gcc atg gcc cag gtc aag ctg cag gag tca ggg act  192
Met Ala Gln Pro Ala Met Ala Gln Val Lys Leu Gln Glu Ser Gly Thr
     50                  55                  60
```

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gaa ctg gca aag cct ggg gcc gca gtg aag atg tcc tgc aag gct tct  240
Glu Leu Ala Lys Pro Gly Ala Ala Val Lys Met Ser Cys Lys Ala Ser
 65                  70                  75                  80

ggc tac acc ttt act gac tac tgg atg cac tgg gtt aaa cag agg cct  288
Gly Tyr Thr Phe Thr Asp Tyr Trp Met His Trp Val Lys Gln Arg Pro
                 85                  90                  95

gga cag ggt ctg gaa tgg att gga tac att aat cct aac act gct tat  336
Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Asn Thr Ala Tyr
            100                 105                 110

act gac tac aat cag aaa ttc aag gac aag gcc aca ttg act gca gac  384
Thr Asp Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp
        115                 120                 125

aaa tcc tcc agc aca gcc tac atg caa ctg cgc agc ctg acg tct gag  432
Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Arg Ser Leu Thr Ser Glu
    130                 135                 140

gat tct gca gtc tat tac tgt gca aaa aag aca act cag act acg tgg  480
Asp Ser Ala Val Tyr Tyr Cys Ala Lys Lys Thr Thr Gln Thr Thr Trp
145                 150                 155                 160

ggg ttt cct ttt tgg ggc caa ggg acc acg gtc acc gtc tcc tca ggt  528
Gly Phe Pro Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly
                165                 170                 175

gga ggc ggt tca ggc gga ggt ggc tct ggc ggt ggc gga tcg gac att  576
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
            180                 185                 190

gtg ctg acc cag tct cca aaa tcc atg gcc atg tca gtc gga gag agg  624
Val Leu Thr Gln Ser Pro Lys Ser Met Ala Met Ser Val Gly Glu Arg
        195                 200                 205

gtc acc ttg agc tgc aag gcc agt gag aat gtg gat tct ttt gtt tcc  672
Val Thr Leu Ser Cys Lys Ala Ser Glu Asn Val Asp Ser Phe Val Ser
    210                 215                 220

tgg tat caa cag aaa cca ggc cag tct cct aaa ctg ctg ata tac ggg  720
Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Gly
225                 230                 235                 240

gcc tcc aac cgg tac act ggg gtc ccc gat cgc ttc gca ggc agt gga  768
Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ala Gly Ser Gly
                245                 250                 255

tct gga aga gat ttc act ctg acc atc agc agt gtg cag gct gaa gac  816
Ser Gly Arg Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
            260                 265                 270

ctt gca gat tat cac tgt gga cag aat tac agg tat ccg ctc acg ttc  864
Leu Ala Asp Tyr His Cys Gly Gln Asn Tyr Arg Tyr Pro Leu Thr Phe
        275                 280                 285

ggt gct ggc acc aag ctg gaa atc aaa cgg gcg gcc gca gag ctc ggc  912
Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Glu Leu Gly
    290                 295                 300

gga ggt ggc tct atg gca gac gaa gat ctc atc ttc cgc ctg gaa ggc  960
Gly Gly Gly Ser Met Ala Asp Glu Asp Leu Ile Phe Arg Leu Glu Gly
305                 310                 315                 320
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gtt gat ggc ggc cag tcc ccc cga gct ggc cat gat ggt gat tct gat   1008
Val Asp Gly Gly Gln Ser Pro Arg Ala Gly His Asp Gly Asp Ser Asp
                325                 330                 335

ggg gac agc gac gat gag gaa ggt tac ttc atc tgc ccc atc acg gat   1056
Gly Asp Ser Asp Asp Glu Glu Gly Tyr Phe Ile Cys Pro Ile Thr Asp
            340                 345                 350

gac cca agc tcg aac cag aat gtc aat tcc aag gtt aat aag tac tac   1104
Asp Pro Ser Ser Asn Gln Asn Val Asn Ser Lys Val Asn Lys Tyr Tyr
        355                 360                 365

agc aac cta aca aaa agt gag cgg tat agc tcc agc ggg tcc ccg gca   1152
Ser Asn Leu Thr Lys Ser Glu Arg Tyr Ser Ser Ser Gly Ser Pro Ala
    370                 375                 380

aac tcc ttc cac ttc aag gaa gcc tgg aag cac gca atc cag aag gcc   1200
Asn Ser Phe His Phe Lys Glu Ala Trp Lys His Ala Ile Gln Lys Ala
385                 390                 395                 400

aag cac atg ccc gac ccc tgg gct gag ttc cac ctg gaa gat att gcc   1248
Lys His Met Pro Asp Pro Trp Ala Glu Phe His Leu Glu Asp Ile Ala
                405                 410                 415

acc gaa cgt gct act cga cac agg tac aac gcc gtc acc ggg gaa tgg   1296
Thr Glu Arg Ala Thr Arg His Arg Tyr Asn Ala Val Thr Gly Glu Trp
            420                 425                 430

ctg gat gat gaa gtt ctg atc aag atg gca tct cag ccc ttc ggc cga   1344
Leu Asp Asp Glu Val Leu Ile Lys Met Ala Ser Gln Pro Phe Gly Arg
        435                 440                 445

gga gca atg agg gag tgc ttc cgg acg aag aag ctc tcc aac ttc ttg   1392
Gly Ala Met Arg Glu Cys Phe Arg Thr Lys Lys Leu Ser Asn Phe Leu
    450                 455                 460

cat gcc cag cag tgg aag ggc gcc tcc aac tac gtg gcg aag cgc tac   1440
His Ala Gln Gln Trp Lys Gly Ala Ser Asn Tyr Val Ala Lys Arg Tyr
465                 470                 475                 480

atc gag ccc gta gac cgg gat gtg tac ttt gag gac gtg cgt cta cag   1488
Ile Glu Pro Val Asp Arg Asp Val Tyr Phe Glu Asp Val Arg Leu Gln
                485                 490                 495

atg gag gcc aag ctc tgg ggg gag gag tat aat cgg cac aag ccc ccc   1536
Met Glu Ala Lys Leu Trp Gly Glu Glu Tyr Asn Arg His Lys Pro Pro
            500                 505                 510

aag cag gtg gac atc atg cag atg tgc atc atc gag ctg aag gac aga   1584
Lys Gln Val Asp Ile Met Gln Met Cys Ile Ile Glu Leu Lys Asp Arg
        515                 520                 525

ccg ggc aag ccc ctc ttc cac ctg gag cac tac atc gag ggc aag tac   1632
Pro Gly Lys Pro Leu Phe His Leu Glu His Tyr Ile Glu Gly Lys Tyr
    530                 535                 540

atc aag tac aac tcc aac tct ggc ttt gtc cgc gat gac aac atc cgc   1680
Ile Lys Tyr Asn Ser Asn Ser Gly Phe Val Arg Asp Asp Asn Ile Arg
545                 550                 555                 560

ctg acg ccg cag gcc ttc agc cac ttc act ttt gag cgt tcc ggc cat   1728
Leu Thr Pro Gln Ala Phe Ser His Phe Thr Phe Glu Arg Ser Gly His
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                    565                 570                 575
cag ctg ata gtg gtg gac atc cag gga gtt ggg gat ctc tac act gac   1776
Gln Leu Ile Val Val Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp
            580                 585                 590

cca cag atc cac acg gag acg ggc act gac ttt gga gac ggc aac cta   1824
Pro Gln Ile His Thr Glu Thr Gly Thr Asp Phe Gly Asp Gly Asn Leu
        595                 600                 605

ggt gtc cgc ggg atg gcg ctc ttc ttc tac tct cat gcc tgc aac cgg   1872
Gly Val Arg Gly Met Ala Leu Phe Phe Tyr Ser His Ala Cys Asn Arg
    610                 615                 620

att tgc gag agc atg ggc ctt gct ccc ttt gac ctc tcg ccc cgg gag   1920
Ile Cys Glu Ser Met Gly Leu Ala Pro Phe Asp Leu Ser Pro Arg Glu
625                 630                 635                 640

agg gat gca gtg aat cag aac acc aag ctg ctg caa tca gcc aag acc   1968
Arg Asp Ala Val Asn Gln Asn Thr Lys Leu Leu Gln Ser Ala Lys Thr
                645                 650                 655

atc ttg aga gga aca gag gaa aaa tgt ggg agc ccc cga gta agg acc   2016
Ile Leu Arg Gly Thr Glu Glu Lys Cys Gly Ser Pro Arg Val Arg Thr
            660                 665                 670

ctc tct ggg agc cgg cca ccc ctg ctc cgt ccc ctt tca gag aac tct   2064
Leu Ser Gly Ser Arg Pro Pro Leu Leu Arg Pro Leu Ser Glu Asn Ser
        675                 680                 685

gga gac gag aac atg agc gac gtg acc ttc gac tct ctc cct tct tcc   2112
Gly Asp Glu Asn Met Ser Asp Val Thr Phe Asp Ser Leu Pro Ser Ser
    690                 695                 700

cca tct tcg gcc aca cca cac agc cag aag cta gac cac ctc cat tgg   2160
Pro Ser Ser Ala Thr Pro His Ser Gln Lys Leu Asp His Leu His Trp
705                 710                 715                 720

cca gtc ttc agt gac ctc gat aac atg gca tcc aga gac cat gat cat   2208
Pro Val Phe Ser Asp Leu Asp Asn Met Ala Ser Arg Asp His Asp His
                725                 730                 735

cta gac aac cac cgg gag tct gag aat agt ggg gac agc gga tac ccc   2256
Leu Asp Asn His Arg Glu Ser Glu Asn Ser Gly Asp Ser Gly Tyr Pro
            740                 745                 750

agt gag aag cgg ggt gag ctg gat gac cct gag ccc cga gaa cat ggc   2304
Ser Glu Lys Arg Gly Glu Leu Asp Asp Pro Glu Pro Arg Glu His Gly
        755                 760                 765

cac tca tac agt aat cgg aag tac gag tct gac gaa gac agc ctg ggc   2352
His Ser Tyr Ser Asn Arg Lys Tyr Glu Ser Asp Glu Asp Ser Leu Gly
    770                 775                 780

agc tct gga cgg gta tgt gta gag aag tgg aat ctc ctc aac tcc tcc   2400
Ser Ser Gly Arg Val Cys Val Glu Lys Trp Asn Leu Leu Asn Ser Ser
785                 790                 795                 800

cgc ctc cac ctg ccg agg gct tcg gcc gtg gcc ctg gaa gtg caa agg   2448
Arg Leu His Leu Pro Arg Ala Ser Ala Val Ala Leu Glu Val Gln Arg
                805                 810                 815

ctt aat gct ctg gac ctc gaa aag aaa atc ggg aag tcc att ttg ggg   2496
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```
Leu Asn Ala Leu Asp Leu Glu Lys Lys Ile Gly Lys Ser Ile Leu Gly
            820                 825                 830

aag gtc cat ctg gcc atg gtg cgc tac cac gag ggt ggg cgc ttc tgc   2544
Lys Val His Leu Ala Met Val Arg Tyr His Glu Gly Gly Arg Phe Cys
        835                 840                 845

gag aag ggc gag gag tgg gac cag gag tcg gct gtc ttc cac ctg gag   2592
Glu Lys Gly Glu Glu Trp Asp Gln Glu Ser Ala Val Phe His Leu Glu
    850                 855                 860

cac gca gcc aac ctg ggc gag ctg gag gcc atc gtg ggc ctg gga ctc   2640
His Ala Ala Asn Leu Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu
865                 870                 875                 880

atg tac tcg cag ttg cct cat cac atc cta gcc gat gtc tct ctg aag   2688
Met Tyr Ser Gln Leu Pro His His Ile Leu Ala Asp Val Ser Leu Lys
                885                 890                 895

gag aca gaa gag aac aaa acc aaa gga ttt gat tac tta cta aag gcc   2736
Glu Thr Glu Glu Asn Lys Thr Lys Gly Phe Asp Tyr Leu Leu Lys Ala
            900                 905                 910

gct gaa gct ggc gac agg cag tcc atg atc cta gtg gcg cga gct ttt   2784
Ala Glu Ala Gly Asp Arg Gln Ser Met Ile Leu Val Ala Arg Ala Phe
        915                 920                 925

gac tct ggc cag aac ctc agc ccg gac agg tgc caa gac tgg cta gag   2832
Asp Ser Gly Gln Asn Leu Ser Pro Asp Arg Cys Gln Asp Trp Leu Glu
    930                 935                 940

gcc ctg cac tgg tac aac act gcc ctg gag atg acg gac tgt gat gag   2880
Ala Leu His Trp Tyr Asn Thr Ala Leu Glu Met Thr Asp Cys Asp Glu
945                 950                 955                 960

ggc ggt gag tac gac gga atg cag gac gag ccc cgg tac atg atg ctg   2928
Gly Gly Glu Tyr Asp Gly Met Gln Asp Glu Pro Arg Tyr Met Met Leu
                965                 970                 975

gcc agg gag gcc gag atg ctg ttc aca gga ggc tac ggg ctg gag aag   2976
Ala Arg Glu Ala Glu Met Leu Phe Thr Gly Gly Tyr Gly Leu Glu Lys
            980                 985                 990

gac ccg cag aga tca ggg gac ttg tat acc cag gca gca gag gca gcg   3024
Asp Pro Gln Arg Ser Gly Asp Leu Tyr Thr Gln Ala Ala Glu Ala Ala
        995                 1000                1005

atg gaa gcc atg aag ggc cga ctg gcc aac cag tac tac caa aag gct   3072
Met Glu Ala Met Lys Gly Arg Leu Ala Asn Gln Tyr Tyr Gln Lys Ala
    1010                1015                1020

gaa gag gcc tgg gcc cag atg gag gag taa                          3102
Glu Glu Ala Trp Ala Gln Met Glu Glu
1025                1030
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<210> 6
<211> 1033
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: pMT-Ki-4 (scFv)-eEF-2K ORF

<400> 6

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Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
 1               5                  10                  15
Ala Gln Pro Ala Met Ala Met Gly His His His His His His His His
            20                  25                  30
His His Ser Ser Gly His Ile Asp Asp Asp Asp Lys His Met Lys Leu
        35                  40                  45
Met Ala Gln Pro Ala Met Ala Gln Val Lys Leu Gln Glu Ser Gly Thr
    50                  55                  60
Glu Leu Ala Lys Pro Gly Ala Ala Val Lys Met Ser Cys Lys Ala Ser
65                  70                  75                  80
Gly Tyr Thr Phe Thr Asp Tyr Trp Met His Trp Val Lys Gln Arg Pro
                85                  90                  95
Gly Gln Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Asn Thr Ala Tyr
            100                 105                 110
Thr Asp Tyr Asn Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Ala Asp
        115                 120                 125
Lys Ser Ser Ser Thr Ala Tyr Met Gln Leu Arg Ser Leu Thr Ser Glu
    130                 135                 140
Asp Ser Ala Val Tyr Tyr Cys Ala Lys Lys Thr Thr Gln Thr Thr Trp
145                 150                 155                 160
Gly Phe Pro Phe Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly
                165                 170                 175
Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile
            180                 185                 190
Val Leu Thr Gln Ser Pro Lys Ser Met Ala Met Ser Val Gly Glu Arg
        195                 200                 205
Val Thr Leu Ser Cys Lys Ala Ser Glu Asn Val Asp Ser Phe Val Ser
    210                 215                 220
Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Gly
225                 230                 235                 240
Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Ala Gly Ser Gly
                245                 250                 255
Ser Gly Arg Asp Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp
            260                 265                 270
Leu Ala Asp Tyr His Cys Gly Gln Asn Tyr Arg Tyr Pro Leu Thr Phe
        275                 280                 285
Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg Ala Ala Ala Glu Leu Gly
    290                 295                 300
Gly Gly Gly Ser Met Ala Asp Glu Asp Leu Ile Phe Arg Leu Glu Gly
305                 310                 315                 320
Val Asp Gly Gly Gln Ser Pro Arg Ala Gly His Asp Gly Asp Ser Asp
                325                 330                 335
Gly Asp Ser Asp Asp Glu Glu Gly Tyr Phe Ile Cys Pro Ile Thr Asp
            340                 345                 350
Asp Pro Ser Ser Asn Gln Asn Val Asn Ser Lys Val Asn Lys Tyr Tyr
        355                 360                 365
Ser Asn Leu Thr Lys Ser Glu Arg Tyr Ser Ser Ser Gly Ser Pro Ala
    370                 375                 380
Asn Ser Phe His Phe Lys Glu Ala Trp Lys His Ala Ile Gln Lys Ala
385                 390                 395                 400
Lys His Met Pro Asp Pro Trp Ala Glu Phe His Leu Glu Asp Ile Ala
                405                 410                 415
Thr Glu Arg Ala Thr Arg His Arg Tyr Asn Ala Val Thr Gly Glu Trp
            420                 425                 430
Leu Asp Asp Glu Val Leu Ile Lys Met Ala Ser Gln Pro Phe Gly Arg
        435                 440                 445
Gly Ala Met Arg Glu Cys Phe Arg Thr Lys Lys Leu Ser Asn Phe Leu
    450                 455                 460
His Ala Gln Gln Trp Lys Gly Ala Ser Asn Tyr Val Ala Lys Arg Tyr
465                 470                 475                 480
Ile Glu Pro Val Asp Arg Asp Val Tyr Phe Glu Asp Val Arg Leu Gln
                485                 490                 495
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Met Glu Ala Lys Leu Trp Gly Glu Glu Tyr Asn Arg His Lys Pro Pro
500 505 510
Lys Gln Val Asp Ile Met Gln Met Cys Ile Ile Glu Leu Lys Asp Arg
515 520 525
Pro Gly Lys Pro Leu Phe His Leu Glu His Tyr Ile Glu Gly Lys Tyr
530 535 540
Ile Lys Tyr Asn Ser Asn Ser Gly Phe Val Arg Asp Asp Asn Ile Arg
545 550 555 560
Leu Thr Pro Gln Ala Phe Ser His Phe Thr Phe Glu Arg Ser Gly His
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Gln Leu Ile Val Val Asp Ile Gln Gly Val Gly Asp Leu Tyr Thr Asp
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Pro Gln Ile His Thr Glu Thr Gly Thr Asp Phe Gly Asp Gly Asn Leu
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Gly Val Arg Gly Met Ala Leu Phe Phe Tyr Ser His Ala Cys Asn Arg
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Ile Cys Glu Ser Met Gly Leu Ala Pro Phe Asp Leu Ser Pro Arg Glu
625 630 635 640
Arg Asp Ala Val Asn Gln Asn Thr Lys Leu Leu Gln Ser Ala Lys Thr
645 650 655
Ile Leu Arg Gly Thr Glu Glu Lys Cys Gly Ser Pro Arg Val Arg Thr
660 665 670
Leu Ser Gly Ser Arg Pro Pro Leu Leu Arg Pro Leu Ser Glu Asn Ser
675 680 685
Gly Asp Glu Asn Met Ser Asp Val Thr Phe Asp Ser Leu Pro Ser Ser
690 695 700
Pro Ser Ser Ala Thr Pro His Ser Gln Lys Leu Asp His Leu His Trp
705 710 715 720
Pro Val Phe Ser Asp Leu Asp Asn Met Ala Ser Arg Asp His Asp His
725 730 735
Leu Asp Asn His Arg Glu Ser Glu Asn Ser Gly Asp Ser Gly Tyr Pro
740 745 750
Ser Glu Lys Arg Gly Glu Leu Asp Asp Pro Glu Pro Arg Glu His Gly
755 760 765
His Ser Tyr Ser Asn Arg Lys Tyr Glu Ser Asp Glu Asp Ser Leu Gly
770 775 780
Ser Ser Gly Arg Val Cys Val Glu Lys Trp Asn Leu Leu Asn Ser Ser
785 790 795 800
Arg Leu His Leu Pro Arg Ala Ser Ala Val Ala Leu Glu Val Gln Arg
805 810 815
Leu Asn Ala Leu Asp Leu Glu Lys Lys Ile Gly Lys Ser Ile Leu Gly
820 825 830
Lys Val His Leu Ala Met Val Arg Tyr His Glu Gly Gly Arg Phe Cys
835 840 845
Glu Lys Gly Glu Glu Trp Asp Gln Glu Ser Ala Val Phe His Leu Glu
850 855 860
His Ala Ala Asn Leu Gly Glu Leu Glu Ala Ile Val Gly Leu Gly Leu
865 870 875 880
Met Tyr Ser Gln Leu Pro His His Ile Leu Ala Asp Val Ser Leu Lys
885 890 895
Glu Thr Glu Glu Asn Lys Thr Lys Gly Phe Asp Tyr Leu Leu Lys Ala
900 905 910
Ala Glu Ala Gly Asp Arg Gln Ser Met Ile Leu Val Ala Arg Ala Phe
915 920 925
Asp Ser Gly Gln Asn Leu Ser Pro Asp Arg Cys Gln Asp Trp Leu Glu
930 935 940
Ala Leu His Trp Tyr Asn Thr Ala Leu Glu Met Thr Asp Cys Asp Glu
945 950 955 960
Gly Gly Glu Tyr Asp Gly Met Gln Asp Glu Pro Arg Tyr Met Met Leu
965 970 975
Ala Arg Glu Ala Glu Met Leu Phe Thr Gly Gly Tyr Gly Leu Glu Lys
980 985 990
Asp Pro Gln Arg Ser Gly Asp Leu Tyr Thr Gln Ala Ala Glu Ala Ala

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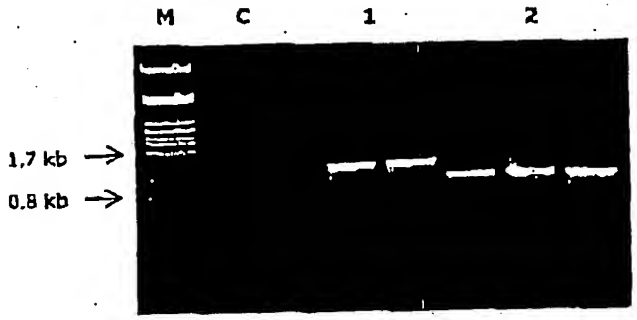
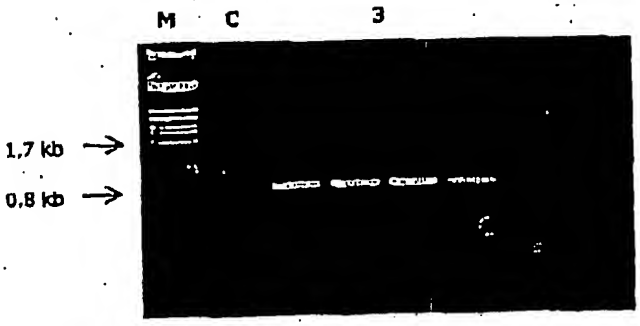
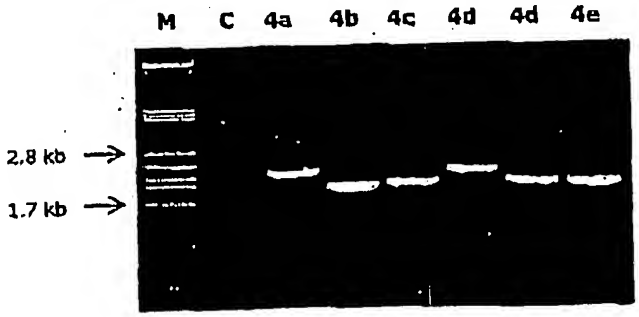
Fig. 1







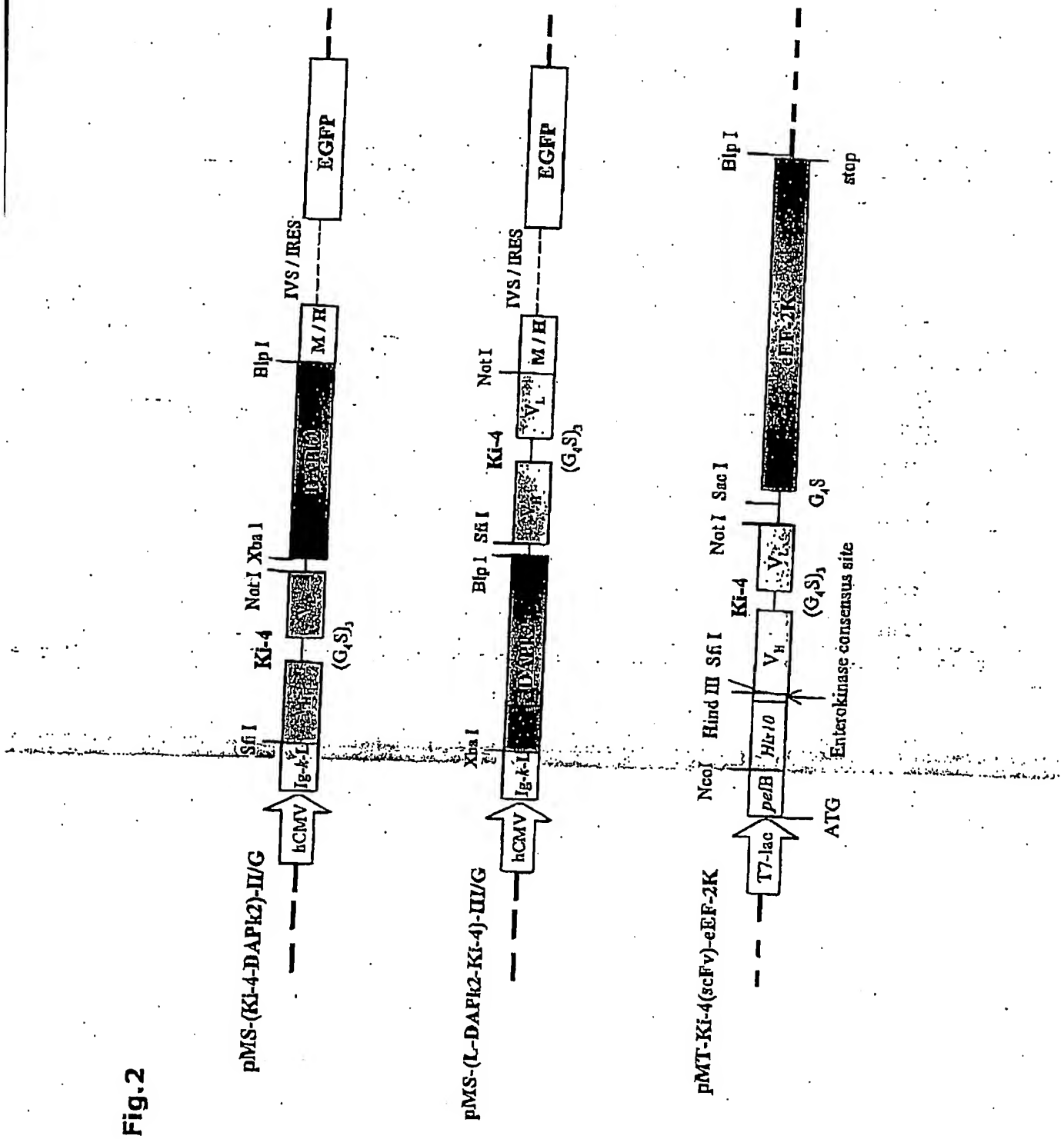
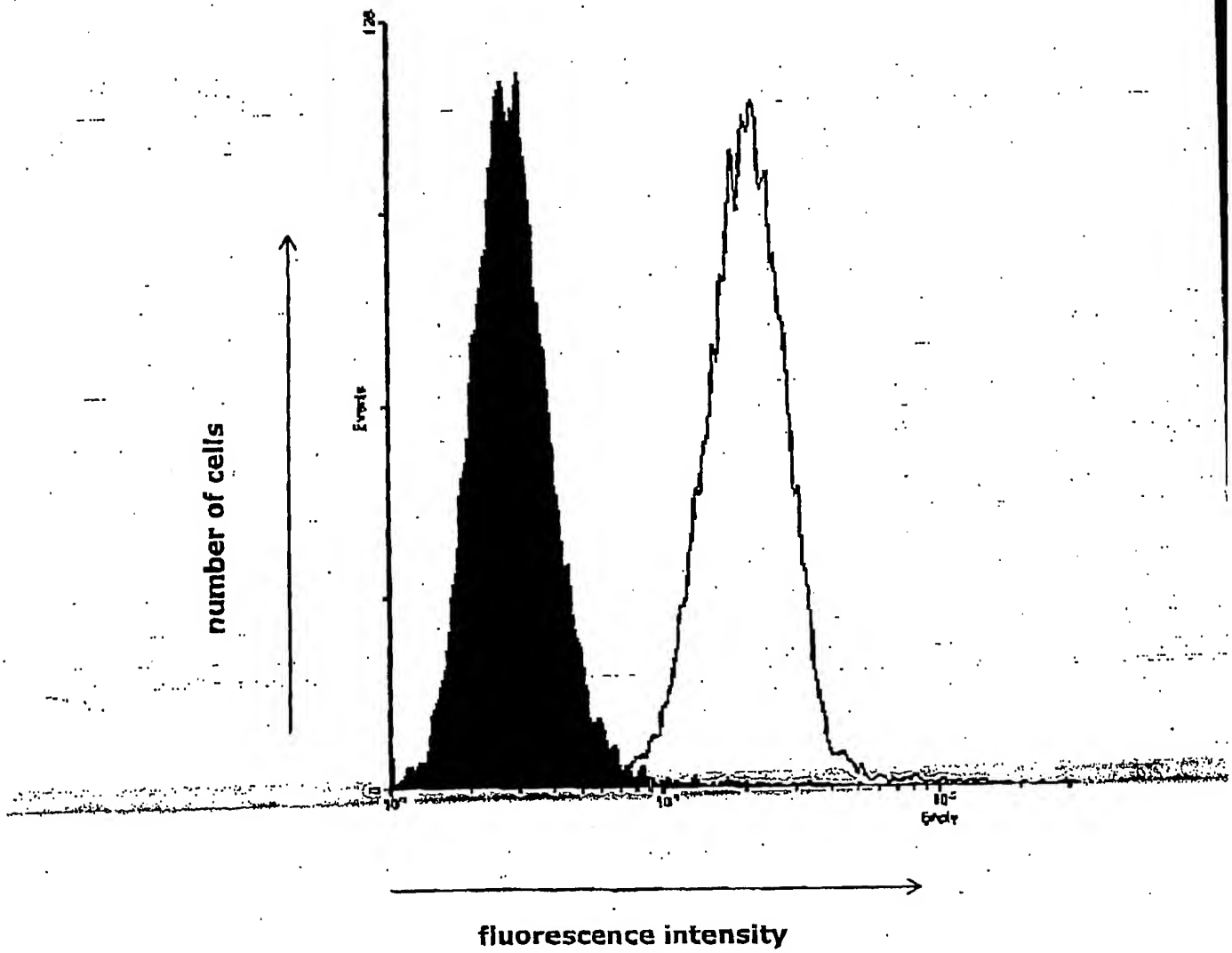


Fig.2

Fig.3



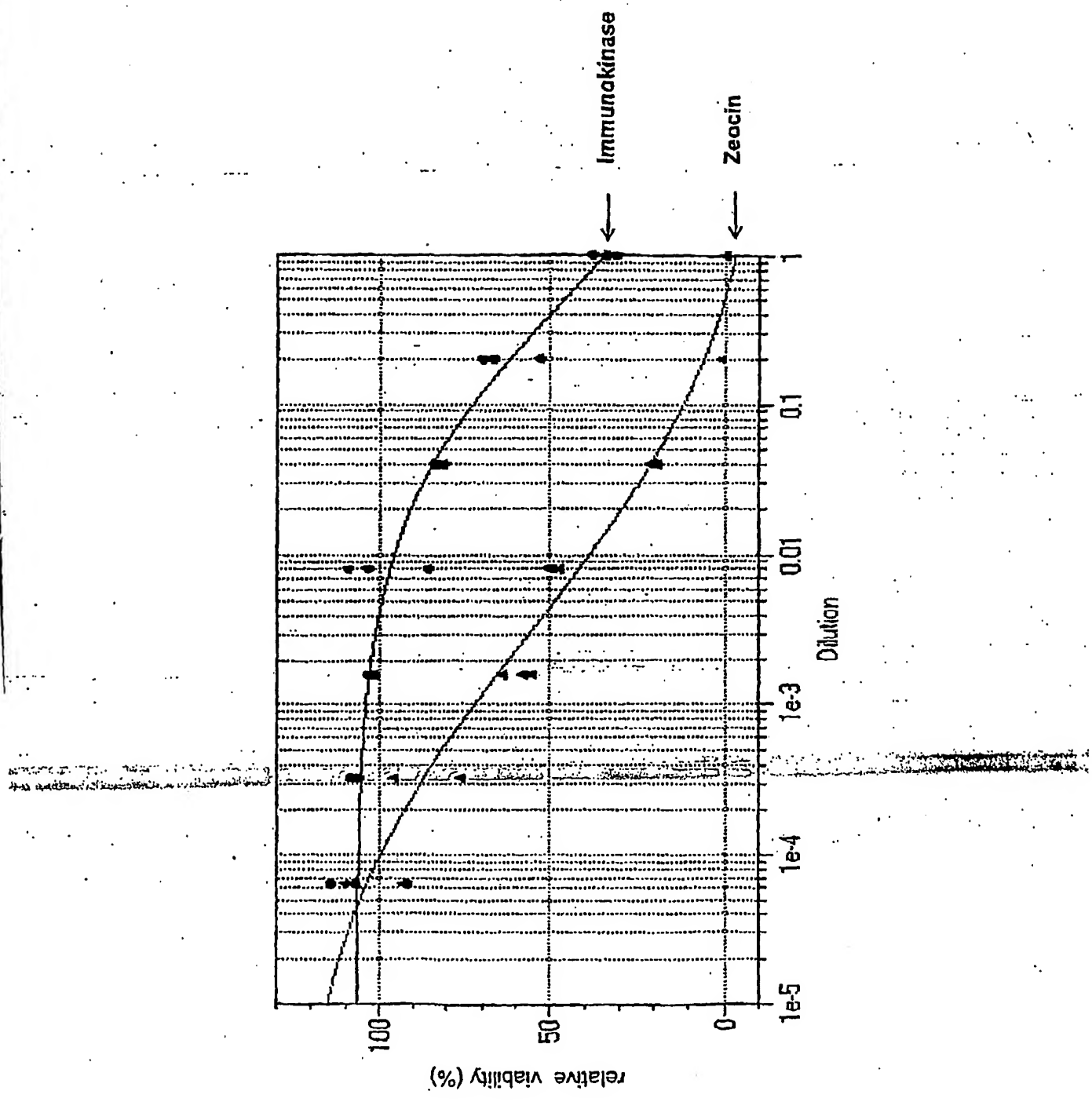


Fig.4